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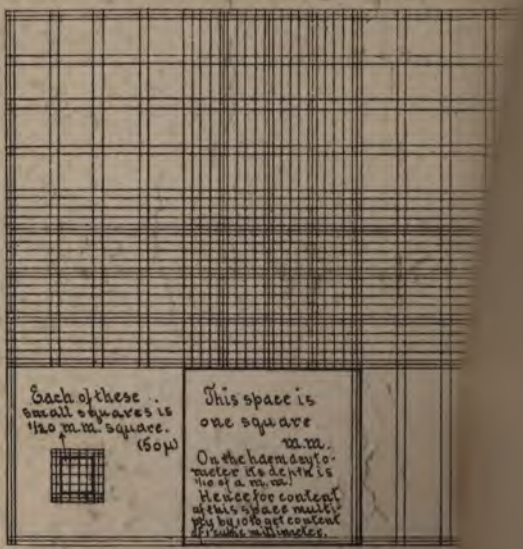

The ruling of the Turck disc. Explanation
the first place, we have the square which inclose
face. This is made up of nine squares, each 1 mm
the squares to use in connection with leukocy
pipette. They may be termed the large
square which can be found are those
double-ruled lines in the center; the
never used for any purpose. Whe
which are found in groups of si
are intended. These squares a
count of red cells. Another
ruled large square in each
space equal to sixteen sm
frame-like space surroun
or thirty-six in all. Th
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left-hand double-line c

There are 400 sm
there are nine large
small squares.

TERIOLOGY, BLOOD WORK
MAL PARASITOLOGY

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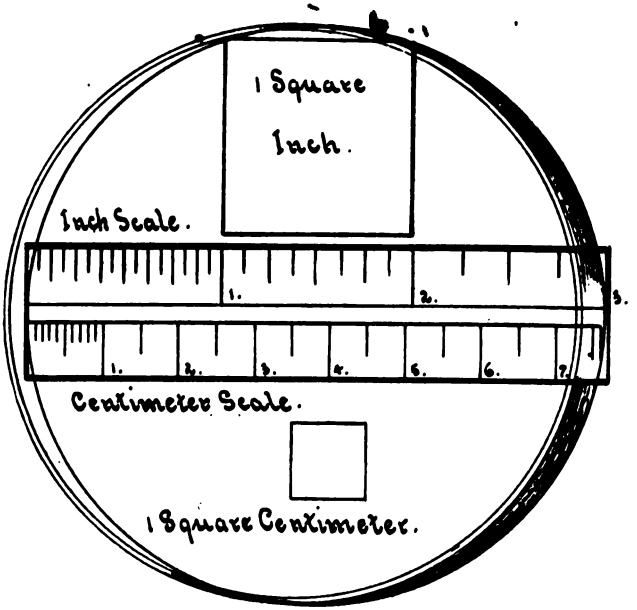

The diameter of the bottom of this Petri dish is 3 inches or 7.5+ centimeters.

The area of a circle is equal to the square of the radius multiplied by π or 22/7.

1 1/2 in. = radius. 1 1/2 × 1 1/2 = 2.25. 2.25 × 22/7 = 7.07 square inches.

3.75 cm. = radius. 3.75 × 3.75 = 14.06. 14.06 × 22/7 = 44.1 square centimeters.

Number of bacterial colonies in 1 sq. in. averages, approximately, 75. Number in 7.07 sq. in. = 530.

Number of bacterial colonies in 1 sq. cm. averages, approximately, 12. Number in 44.1 sq. cm. = 528.

In a microscopic field, if the diameter were 8 small squares (1/20 mm.), the radius would be 4 small squares and the area of such a round field would be 4 × 4 = 16 × 22/7 = 50+. Such a field would contain 50 small squares.

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PRACTICAL BACTERIOLOGY, BLOOD WORK AND ANIMAL PARASITOLOGY

STITT

PRACTICAL

Bacteriology, Blood Work

AND

Animal Parasitology

INCLUDING

Bacteriological Keys, Zoological Tables and Explanatory Clinical Notes

BY

E. R. STITT, A. B., Ph. G., M. D.

SURGEON, U. S. NAVY; GRADUATE LONDON SCHOOL OF TROPICAL MEDICINE; ASSOCIATE PROFESSOR OF MEDICAL ZOOLOGY, PHILIPPINE MEDICAL SCHOOL; FORMERLY INSTRUCTOR IN BACTERIOLOGY AND TROPICAL MEDICINE, U. S. NAVAL MEDICAL SCHOOL, AND LECTURER IN TROPICAL MEDICINE, JEFFERSON MEDICAL COLLEGE

SECOND EDITION, REVISED AND ENLARGED

WITH 91 ILLUSTRATIONS

PHILADELPHIA

P. BLAKISTON'S SON & CO.

1012 WALNUT STREET

1911

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Printed by
The Maple Press
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PREFACE TO THE SECOND EDITION.

THE fact of the necessity for a second edition of this manual of laboratory and clinical diagnosis in a little more than a year would indicate that the original arrangement of material should be adhered to.

Each section of the book has been carefully revised and much new matter added. In particular has that part of the book relating to animal parasitology been rewritten and almost doubled in extent, and a chapter on Poisonous Snakes added. In the chapter on "Practical Methods in Immunity" the most recent advances in the Wasserman test and practical agglutination methods have been incorporated as well as a brief discussion of the question of Anaphylaxis.

The section on "Clinical Bacteriology and Animal Parasitology of the Various Body Fluids and Organs" has been revised to meet the most recent advances in clinical diagnosis. This section not only answers as a cross index to the importance of the various bacteria and animal parasites in practical clinical work, but gives a concise, practical statement as to how to proceed in the examination of various secretions and excretions. This information is difficult to obtain in the larger works on clinical diagnosis by reason of its being taken up under many different headings.

A method is given for the making of differential counts in the same preparation as that for making the leukocyte count which has the advantages of accuracy and the saving of time.

Several new illustrations have been added—the one of poisonous snakes has been taken from Stejneger's report.

The plan of making this little volume a practical one has been continued in the second edition; theoretical considerations have been brought out only when necessary to a proper understanding of some recent or difficult laboratory method.

The very elementary considerations and definitions have not been given because in order to present a compact and at the same time a practical working guide it has been necessary to eliminate that which

seemed least essential. Furthermore, instruction in biological science is now a part of the requirements of candidates for admission to the various medical schools.

At the request of many who have found the book of assistance I have added an outline of those methods in the chemical examination of urine and gastric contents which have seemed to me to be most essential in the making of diagnoses. In the tropics I have found the determinations of total nitrogen and nitrogen eliminated as ammonia to be exceedingly valuable in diagnosis. Methods for such determinations, as elaborated by Assistant Surgeon E. W. Brown, U. S. Navy, of the U. S. Naval Medical School, have proven satisfactory and have been incorporated in this section which is to be found in the Appendix.

Every effort has been made to keep the book within the limits of a pocket manual.

Owing to my absence from the United States I have to thank Dr. Charles S. Butler for correcting the proof. For the revision of the index I am indebted to Mr. John P. Griest.

E. R. S.

CAVITE, P I.

PREFACE TO THE FIRST EDITION.

WHILE a member of the Naval Examining Board and examiner in bacteriology and clinical microscopy, I have during the past six years had an opportunity to judge of the qualifications of several hundred graduates of the various medical schools of the country from the standpoint of practical application in the laboratory of that which they had learned as undergraduates.

More particularly I have made it a point to ascertain from the successful candidates, while under instruction at the Naval Medical School, the features of their laboratory courses, which had seemed to them most practical; such methods being subsequently tested in our own class work.

As a result I have endeavored to incorporate in this manual methods which have been submitted to the criticism of postgraduate students from all the leading medical schools of the country, and which have been considered by them adapted to the requirements of practical, speedy, and satisfactory clinical laboratory diagnosis.

For the laboratory worker the most valuable asset is common sense and he must be able to bring to mind the possibilities of the production of various artefacts and results from trivial errors in technic. It has been my object to point out where such mistakes may arise, the reasons for obtaining results differing from those ordinarily obtained and the means employed to eliminate as far as possible such results.

We are too apt to neglect the trivial details of stains, reaction of media, and the like, yet it is only when every detail of technic has been rigidly carried out that we are in a position to judge of the significance of an object observed in a microscopical preparation.

In bacteriology, candidates were frequently able to give the cultural and morphological characteristics of all the important pathogenic organisms, yet when it was required of them to outline the procedure by which they would differentiate members of the typhoid-colon groups when encountered in a plate made from feces, the problem appeared

to them impossible. They possessed the information, but did not know how to apply it.

In practical work, organisms can only be separated culturally by the use of Keys and for this reason Keys are given at the beginning of each division of bacteria. These enable one to quickly place the organism isolated in its respective group. Only methods of differentiation which are applicable in a physician's private laboratory are given. Practical methods for making the final identification by agglutination or other immunity tests are described. Technic for immunizing animals to furnish such sera is given in detail.

The giving of the cultural characteristics in a systematic tabulated Key gives space in the notes for presenting the salient points in the pathological and epidemiological aspects of each organism.

I have endeavored to give a scientific yet practical classification of the important pathogenic moulds, a subject about which there exists greater confusion in the minds of students than for any other. In the nomenclature I have followed Gedoelst's "Les Champignons Parasites."

In the chapter on Media Making, it is believed that anyone after reading this section and following the instructions will be able to satisfactorily and without the adjuncts of a large laboratory make any kind of media. The directions as to titrations are given in detail because it is beginning to be recognized that reaction of media in bacteriology is of as great importance as staining is in blood work.

The section on Blood Work is practical and gives a method for making a Romanowsky stain which is quick and reliable. The chapter on Normal and Pathological Blood gives in a few pages the more important points to be borne in mind in considering a possible diagnosis.

While there is no difference between the laboratory requirements of medical work in the tropics and that in temperate climates, unless by reason of such measures of diagnosis being indispensable in the tropics, it has, however, been my endeavor to treat every tropical question, whether in blood work, bacteriology, or animal parasitology, in a more complete way than is usual in manuals of this character. Therefore it is believed that this little book will be of great service to the laboratory worker in the tropics.

It is only from working under Doctor Charles W. Stiles in his course of laboratory instruction in Animal Parasitology in the United States Naval Medical School that I feel justified in presenting a concise out-

line of the subjects in medical zoology which appear to me to be most important for the physician.

The system of arranging tables, showing the families, genera, etc., in which each species belongs will, it is believed, greatly simplify the matter of classification for the medical student. The points given under each parasite are believed to be practical ones. When a parasite has only been reported for man two or three times, very little space is given to it.

Part IV summarizes the various infections which may be found in different organs or excretions of the body and embraces both bacterial and animal parasites. Practical methods for examining material are also given.

The chapter on Immunity, in which the theoretical side is immediately illustrated by the practical application will tend to simplify this bug-bear of the medical student.

The illustrations have been selected with a view to bringing out points which are difficult to state briefly in the text, and furthermore they have been grouped together so that comparison of similar parasites is possible without turning from page to page.

I have in particular to thank Hospital Steward Ebeling of the Navy for his care in bringing out such details.

By reason of the authority of Braun, it has been considered sufficient to give in the tables only the proper zoological name of the parasite as given in the 1908 German edition. The synonyms have been omitted for consideration of space.

The works chiefly consulted in addition to that of Braun have been:

Albutt's System of Medicine; Osler's System of Medicine; Muir and Ritchie's Bacteriology; Mense's Tropenkrankheiten; Blanchard's Les Moustiques; Guiart and Grimbert's Diagnostic; Ehrlich's Studies in Immunity; Stephens and Christopher's Practical Study of Malaria; Daniel's Laboratory Studies in Tropical Medicine; Manson's Tropical Diseases; Gedoelst's Les Champignons Parasites; Neveu-Lemaire Parasitologie Humaine; Chester's Determinative Bacteriology; Lehmann and Neumann's Bacteriology.

E. R. S.

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BACTERIOLOGY, BLOOD-WORK AND ANIMAL PARASITOLOGY.

CHAPTER I.

APPARATUS.

THE MICROSCOPE.

THE most important piece of apparatus for the laboratory worker is the microscope. Very satisfactory microscopes can be purchased in this country. Instruments of standard German make are in use in many laboratories and appear to give general satisfaction. It is impossible to do good microscopical work unless the microscope gives and continues to give good definition and the working parts remain firm. Folding microscope stands are now made which are perfectly satisfactory.

A mechanical stage is almost a necessity in connection with blood-work and its use is advantageous in bacterial preparations. For the study of tissue sections the moving of the slide with the fingers is preferable. Therefore, the mechanical stage should be capable of ready attachment or removal. For the examination of colonies growing in Petri dishes we also use the stage unencumbered with the mechanical stage. A triple or quadruple nose-piece, according to the number of objectives used, is also indispensable. To meet the demands of clinical microscopy there should be three objectives, preferably a 16 mm. (2/3 in.), a 4 mm. (1/6 in.) and a 2 mm. (1/12 in.) homogeneous oil immersion. The Zeiss AA is a 17 mm. objective, and the Leitz No. 3, an 18 mm. one. The Zeiss D is about 4.2 mm. and the Leitz No. 6, a 4.4 mm. A dust-proof quadruple nose-piece with four objectives will be found a great convenience (in addition to the 2/3-in. and 1/12-in. objectives, a 1/4-in. for urine and blood counting, with a 1/8-in. for examining hanging-drop preparations and for quick examination of blood smears). An apochromatic objective costs about three times as much as an achromatic one and, except in photographic work, has little if any advantage.

As regards oculars (eye-pieces) a No. 2 and a No. 4 will best meet the requirements. For high magnification a No. 8 may be of service. The Zeiss oculars are numbered according to the amount they increase the magnification given by the objective; thus a No. 2 increases the magnification, given by the objective alone, twice; a No. 8, eight times. Some oculars are classified according to the equivalent focal distance, and are referred to as 1/2-in., 1-in., and 2-in. oculars.

The oculars in common use are known as negative oculars, by which is meant an ocular in which the lower lens (collective) assists in forming the real inverted image which is focused at the level of the diaphragm within the ocular. When using a disc micrometer, it is supported by this diaphragm, and the outlines of the image are cut by the rulings on the glass disc, and so we are enabled to measure the size of the object examined. The measurement of various bacteria, blood-cells, and parasites is exceedingly simple and assists greatly in the study of bacteria, and is indispensable in work in animal parasitology. (For details of micrometry see section on blood-work.) When an ocular is termed positive, it refers to an ocular which acts as a simple microscope in magnifying the image, the image being formed entirely by the objective and being located below the ocular.

Objectives are usually designated by their equivalent focal distance. It is important to remember that the equivalent focal distance does not represent the working distance of an objective, by which is meant the distance from the upper surface of the cover-glass to the lower surface of the objective. Thus a 1/4-in. objective may have to be approached to the object so that the distance intervening may be only 1/6 in. or even less. This explains the frequent inability to focus an object when a high-power dry objective (1/6 in. or 1/8 in.) is used with a rather thick cover-glass—the objective possibly having a short working distance, so that the thickness of the cover-glass does not allow of any free working distance.

Instrument makers generally specify the thickness of cover-glass to be used with a certain tube length, but as a practical matter it will be found convenient to use No. 1 (very thin) cover-glasses. The principal objection to these is that they are more fragile than the No. 2, but with a little practice in cleaning cover-glasses this is negligible. Immersion lenses are less affected than dry lenses by the question of a certain thickness of cover-glasses for a certain tube length.

One of the most fruitful causes of the crushing of microscopical objects and the overlying cover-glass or, what is far more important, the breaking of the cover-glass of a hanging-drop preparation and consequent risk of infection is the attempt to focus with the fine adjustment. It should be an invariable rule for the worker to bring his objective practically into contact with the upper surface of the cover-glass, then using the coarse adjustment (rack and pinion) to slowly elevate the objective, looking through the eye-piece at the same time. In other words, obtain focus with the coarse adjustment and maintain it with the fine adjustment (micrometer screw). The fine adjustment should only be used after the focus is obtained. It saves time and disappointment to make a preliminary examination of a preparation requiring the high dry or immersion lens with a low power (2/3 in.) before employing the higher power; in this way we locate or center a suitable field for study.

It will be observed that objectives frequently have their numerical aperture marked on them. This is expressed by the letters N.A. From a practical stand-point this gives the relative proportion of the rays which proceeding from an object can enter the lens of the objective and form the image. Of course, the greater the number of rays, the greater the N.A., the better the definition, and consequently the better the objective. Immersion oil, having the same index of refraction (1.52) as glass, would not deflect rays coming from the object and so prevent their entering the objective, as would be the case if we used a dry objective with an intervening air space. In this case a portion of the rays would be turned aside by the difference in the refractive index of air. As a rule, the higher the numerical aperture, the better the objective and the less the working distance. In blood counting, the cover-glass being comparatively thick, it may happen that with a 1/6-in. of high numerical aperture there may not be sufficient working distance to bring the blood-cells into focus, which could be done with an objective of lower numerical aperture. Consequently, we must always consider the matter of working distance as well as that of numerical aperture. The skill of the optician, however, can obviate this defect in an objective of high numerical aperture so that it may combine the qualities of perfect definition with sufficient working distance.

An important matter in the use of the microscope is to get all the details possible with a low power before using a higher power. This,

of course, does not apply to a bacterial preparation where it is necessary to use a 1/12-in. or a high-power dry lens. With tissue sections, however, it is not only advisable to begin the study with the lowest power, but even an examination with the unaided eye or with a magnifying glass, before using the microscope, will give a surprising amount of information.

It is advisable to cultivate the use of both eyes in doing microscopical work. When using one eye the other should be kept open with accommodation relaxed. It is this squinting of the unemployed eye which so often fatigues. A strip of cardboard 4 or 5 inches long, with an opening to fit over the tube of the microscope, leaving the other end to block the vision of the unused eye, will prevent the strain. This apparatus can be purchased in vulcanite.

A warm stage for the study of living protozoa may be extemporized by taking a piece of copper about the size of the stage and with a strip projecting out anteriorly for 5 or 6 inches. The under surface of the plate is covered with flannel and a hole about 1 inch in diameter cut out of the center. The proper amount of heat is applied by a flame impinging on the tongue-like projection of the copper plate.

Direct sunlight or excessively bright light is to be avoided. If such conditions must exist a white shade or muslin curtain drawn across the window is a necessity. Light from the north and from a white cloud is the most desirable. South of the equator a southern light. In the tropics a piece of plate glass fitted into the lower part of a wire screen frame gives good lighting, keeps out dust, and does not interfere greatly with the circulation of the air.

The technic in connection with proper illumination is probably more important than any other point; unless the light is utilized to the best advantage, the best results cannot be obtained. In examining fresh blood preparations or hanging drops the concave mirror should be used and the light almost shut off by the iris diaphragm so as to give a contour picture. In examining a stained blood or bacterial preparation, the Abbe condenser should be properly focused so as to best illuminate the stained film. In many instruments set-screws are provided which check the elevation of the Abbe condenser when the proper focus is reached. Inasmuch as the light from the condenser should come to a focus exactly level with the object studied, it is evident that a fixed position for the condenser would not answer when slides of different thickness

were used. Always use the plane mirror when examining stained bacterial or blood films, as a color image is desired. Ordinarily in examining tissue sections, the Abbe condenser should either be put out of focus by racking down or by the use of the concave mirror and the narrowing of the aperture of the iris diaphragm. Swing-out condensers are now made which are very convenient. The proper employment of illumination only comes with experience, and one should continue to manipulate his mirrors, diaphragm, and condenser until the best result is obtained. Then study the specimen.

Very valuable information, especially as regards the detection of Treponema in material from hard chancres or mucous patches, may be obtained by the use of dark ground illumination. There are many different types of apparatus for this purpose.

The bacteria or spirochætes are intensely illuminated and show as brilliant silvery objects in contrast to the dark background.

As a source of illumination we may use a small arc-lamp or a Nernst lamp or an incandescent gas lamp.

In making preparations the slides and cover-slips should be scrupulously clean and the material thinly spread out and free of bubbles.

Apparatus for Sterilization.

For the purpose of sterilizing glassware, media, and old cultures there are three methods ordinarily employed. The hot-air sterilizer, in which a temperature of about 150° C. is maintained for one hour, is ordinarily used for the sterilization of Petri dishes, test-tubes, pipettes, etc. If the temperature is allowed to go too high, there is danger of charring the cotton plugs and also of causing the development of an empyreumatic oil which makes the plugs unsightly and causes them to stick to the glass. Again we must be careful not to open the door until the temperature has fallen to 60° C., otherwise there is danger of cracking the glassware. Where gas is not obtainable, the hot-air sterilizer is not a very satisfactory apparatus.

The Arnold sterilizer is to be found everywhere and can be used on blue-flame kerosene-oil stoves as readily as with gas burners. The most convenient form, but more expensive, is the Boston Board of Health pattern. The ordinary pattern, with a telescoping outer portion, answers all purposes, however. In the Arnold, sterilization is effected

by streaming steam at 100° C. It is usual to maintain this temperature for fifteen to twenty-five minutes each day for three successive days. The success of this procedure—fractional sterilization—is due to the fact that many spores which were not killed at the first steaming have developed into vegetative forms within twenty-four hours, and when the steam is then applied such forms are destroyed. Experience has shown that all the spores have developed by the time of the third steaming, so that with this final application of heat we secure perfect sterilization.

It is customary to use the Arnold for sterilizing gelatin and milk media, even when the autoclave is at hand, the idea being that the greater heat of the autoclave may interfere with the quality of such media. The most convenient autoclave is the horizontal type, such as is to be found everywhere for the sterilization of surgical dressings. The source of heat may be either gas, the Primus kerosene-oil lamp or steam from an adjacent boiler. During the past year, in the laboratory of the U. S. Naval Medical School, we have been using a dressing sterilizer, made by the American Sterilizer Co., with which it has been possible to most satisfactorily carry out all kinds of sterilization, thus doing away with the use of the Arnold and the hot-air sterilizer. It is impossible to sterilize ordinary fermentation tubes in the autoclave on account of the boiling up of the media and wetting of the plugs. This is still done with the Arnold. By use of the Durham tubes—which are to be preferred, except for gas analysis—sugar media can be thus sterilized.

Should a small bubble remain in the top of the small inverted inner tube after removal from the autoclave, one may make a mark with a grease pencil at the line of the bubble; or, if preferred, the basket of Durham tubes can be heated to boiling for ten minutes in a pan of water or in the Arnold when, after cooling, the bubble will be found to have disappeared.

Glassware will come out from such an autoclave with wrappers as dry and plugs of the test-tubes as stopper-like as could be effected in a hot-air sterilizer.

The objection which exists in the use of some autoclaves, as regards condensation on dressings or apparatus, does not exist in this type. The mechanism, by which the inner and outer chambers are connected and disconnected, and that for vacuum production, rests in the simple turning of a lever from mark to mark. We have been able with a gas

burner to obtain a pressure of 15 pounds in less than ten minutes. In sterilizing test-tubes we place them in small rectangular wire baskets, 6 x 5 x 4 in. These baskets are to be preferred to round ones, as they pack more satisfactorily in the refrigerator used for storing media.

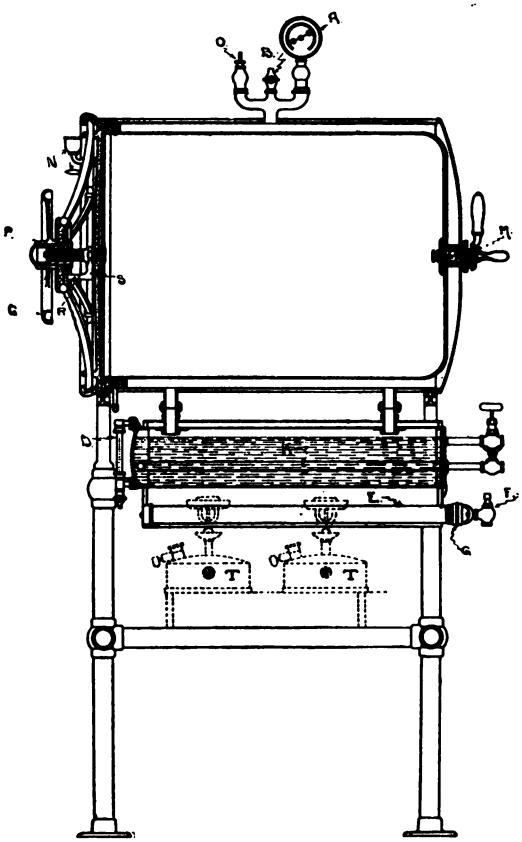

FIG. 1.—Dressing sterilizer showing cylinder containing water (K) heated either by gas or Primus kerosene lamps.

In sterilizing flasks, test-tubes, Petri dishes, throat swabs, pipettes, etc., it has been our custom, after exposing to 20 pounds' pressure for twenty minutes, to produce a vacuum for two or three minutes; then with the steam in the outer jacket for a few minutes to thoroughly dry the articles

in the disinfecting chamber. The valve to the inner chamber is then opened to break the vacuum; the door is now opened, and the articles removed in as dry a state as if they had been in the hot-air sterilizer. Articles, however, can be thoroughly dried without the use of a vacuum, simply allowing the steam to remain in the outer jacket with the steam cut off from the inner chamber.

Pressure and Temperature Table.

5 pounds' pressure,	107.7° C.,	226° F.
10 pounds' pressure,	115.5° C.,	240° F.
15 pounds' pressure,	121.6° C.,	250° F.
20 pounds' pressure,	126.6° C.,	260° F.
25 pounds' pressure,	130.5° C.,	267° F.
30 pounds' pressure,	134.4° C.,	274° F.

All such articles as Petri dishes, pipettes, swabs, etc., are wrapped in cheap quality filter-paper, making a fold and turning in the ends as is done in a druggist's package. Old newspapers answer well for this purpose. The sterile swab can be used for many purposes in the laboratory. They are most easily made by taking a piece of copper wire about 8 inches long, flattening one end with a stroke of a hammer, then twisting a small pledget of plain absorbent cotton around the flattened end. After wrapping, the swabs are sterilized in bunches. We not only use them for getting throat cultures, but in addition for culturing fæces, pus, or other such material. The pus is obtained with a swab, which material is then distributed in a tube of sterile bouillon or water. With the same swab the surface of an agar plate is successively stroked. This method is equally as satisfactory as the German one of using bent glass rods for this purpose. Everyone has encountered the difficulties attendant upon the bending of platinum wires and also the possibility of destroying your organisms by an insufficiently cooled wire.

Cleaning Glassware.

It is a routine in our laboratory for everything to go through the sterilizer at 125° C. before anything else is done. This is a safe rule when dealing with dangerous pathogenic organisms (especially tetanus

and anthrax). As soon as taken out of the sterilizer the contents are emptied, and the tube or dishes placed in a 1% solution of washing soda and boiled. This thoroughly cleans them. As the washing soda slightly raises the boiling-point and also makes the spores more penetrable, it would appear that under ordinary circumstances, it would be sufficient to place all contaminated articles in a dishpan with the soda solution, and boil for at least one hour, not using a preliminary steriliza-

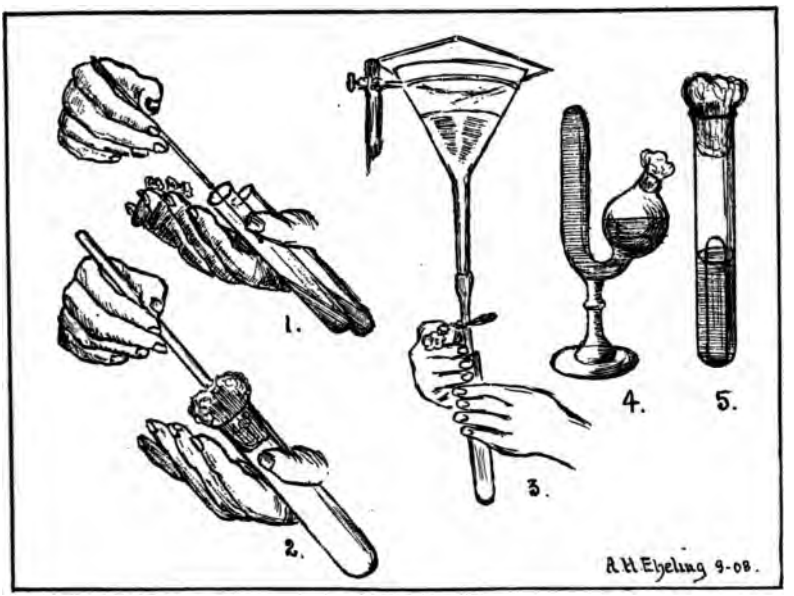

FIG. 2.—1, Inoculation of tubes; 2, plugging of tubes; 3, filling tubes; 4, Smith's fermentation tube; 5, Durham's fermentation tube.

tion in the autoclave. The tubes are now cleaned with a test-tube brush, thoroughly rinsed with tap water and placed in a 1% solution of hydrochloric acid for a few minutes; then rinsed thoroughly in water and placed in test-tube baskets, mouth downward, and allowed to drain over night. Some laboratory workers boil their test-tubes and other glassware in water containing soap or soap powder and, after a thorough rinsing in tap water, drain. Hydrochloric acid should not be used after the soap as it will cause the formation of an unsightly coating difficult to remove. When thoroughly dry they may be plugged and sterilized.

To plug a test-tube, pick out a little pledget of plain absorbent cotton about 2 inches in diameter from a roll. Place it over the center of the tube and with a glass rod push the cotton down the tube about an inch. The cleaning fluid commonly used in laboratories consists of one part each of potassium bichromate and commercial sulphuric acid with ten parts of water. This is an excellent mixture for cleaning old slides, etc., especially when grease or balsam is to be gotten rid of. It is very corrosive, however. An efficient and less corrosive method for cleansing slides and cover-glasses is to leave them over night in an acetic acid alcohol mixture (two parts of glacial acetic acid to one hundred parts of alcohol). After drying and polishing out of this mixture, it is well to pass the slides and cover-glasses through the flame of a Bunsen burner or alcohol lamp to remove every vestige of grease. Ordinarily, rubbing between the thumb and forefinger with soap and water, then drying with an old piece of linen, and finally flaming will yield a perfect surface for making a bacterial preparation.

Concave Slides, Fermentation Tubes.

The concave slide is ordinarily used for making hanging-drop preparations.

To prepare a hanging-drop preparation for the study of motility it is best to place a loopful of the young bouillon culture in the center of the cover-glass; now having applied with a brush a ring of vaseline around the concave depression in the slide we apply the slide as a cover to the cover-glass which latter adheres to the ring of vaseline. The completed

Fig. 3.—Hanging drop, over hollow ground slide. (*Williams.*)

hanging-drop preparation can now be turned over and placed on the stage of the microscope.

A substitute which is equally good may be made by spreading a ring or square of vaseline—smaller than the cover-glass to be used—in the middle of the slide. Then putting a loopful of salt solution in the center of the space, and inoculating with the culture to be studied, we finally cover it with a cover-glass, gently pressing the margins down on the

vaseline. This gives a preparation for the study of motility or agglutination which does not dry out for hours, and is easier to focus upon than the concave slide hanging-drop preparation.

In examining a hanging drop first use a low-power objective and, having brought into focus the margin of the drop as a center line, change to a 1/6- or 1/8-in. objective. By this procedure a thin layer of fluid is brought under the high dry objective instead of the deeper layer in the center of the drop. It is not advisable to use an immersion objective with a hanging-drop preparation.

The fermentation tube with a bulb and closed arm is expensive, difficult to clean, and is easily broken. As a substitute in the study

FIG. 4.—Blood serum coagulating apparatus.

of gas production and in water bacteriology, the Durham tube is to be recommended. Into a test-tube, about 1 x 7 in., we introduce the special sugar media, then drop down a small test-tube (1/2 x 3 in.) with its open end downward. Insert the plug of the large tube and sterilize. During sterilization the fluid enters the mouth of the smaller tube and fills it, and when the medium is subsequently inoculated, if gas forms, it appears in the upper part of the closed end of the smaller tube. For inspissating blood-serum slants a regular inspissator is desirable.

This is nothing more than a double-walled vessel, the space between the walls being filled with water. As a substitute one may take the common rice cooker (double boiler). Fill the outer part with water; and in the inner compartment pack the serum tubes properly slanted

on a piece of wood or a wedge-shaped layer of cotton. Place a weight on the cover of the inner compartment to sink it into the surrounding water, and allow to boil for one or two hours. This same apparatus may be used for their sterilization on two subsequent days, but it is better to sterilize in the autoclave or Arnold. As regards a working desk, it will be found convenient to have an arrangement similar to the ordinary flat-top desk, with a tier of drawers on each side. A block of wood with holes bored in it to contain dropping-bottles may be placed in the upper left-hand drawer. In this way the stains are as accessible as if they encumbered the desk. It is advisable to paint the inside of this drawer black so that the light may not cause the staining reagents to deteriorate.

FIG. 5.—Rice cooker.

A very popular method of preparing the surfaces of laboratory desks, sinks, and tables is the application of the so-called "acid-proofing." This gives an ebony-like finish which is not affected by strong acids.

In using it the surface of the wood must be new (free of any varnish, oil, or paint; if previously so coated the surface must be planed).

Solution 1.

Potassium chlorate,	125.0 gms.
Cupric sulphate,	125.0 gms.
Water,	1000.0 c.c.

Apply two coats of this solution allowing at least 12 hours between applications. When thoroughly dry apply two coats of solution No. 2.

Solution 2.

Aniline oil,	120.0 c. c.
Hydrochloric acid,	180.0 c. c.
Water,	1000.0 c. c.

When the treated surface is thoroughly dry apply one coat of raw linseed oil with a cloth. After this is dry wash with very hot soapsuds.

An aspirating bottle on a shelf elevated two feet, with rubber tubing and glass tip leading to a small aquarium jar or other desk receptacle,

makes a good substitute for a small sink and faucet. A Hoffman screw clamp on the rubber tube controls the flow of water.

Ordinary glass salt cellars will be found very useful, where the watch-glass is employed. They may also be wrapped, sterilized, and used to contain fluids for inoculating, etc.

A glass-topped fruit jar or a specimen jar containing a disinfecting solution for contaminated slides, etc., should be on every working desk. A good solution is that of Harrington (corrosive sublimate, 0.8; commercial HCl, 60.0 c.c.; alcohol, 400.0 c.c.; water, to 1000.0 c.c.).

A very simple method of making a disinfectant similar to lysol is to put one part of cresol or crude carbolic acid and one part of soft soap in a wide-mouthed bottle over night. The resulting compound makes perfect solution with water and a 5 % solution of this will be found at least equal to a 5 % phenol solution. In addition to using as a desk jar disinfectant it is excellent for disinfecting fæces, sputum, etc.

For use in making loops and needles, platinum wire of 26 gauge will be found most suitable. The handle made of glass rod is preferable to the metal ones. One end is fused in the flame and, holding the 3- to 4-in. piece of platinum wire, with forceps, in the same flame, insert the glowing metal into the molten glass.

For making smears from fæces, sputum, and the like, wooden tooth-picks are very convenient; the kind with the spatulate end is preferable.

When gas is obtainable, the maintaining of a constant temperature for the body temperature incubator (38° C.) and the paraffin oven (60° C.) is best secured by the use of some of the various types of thermo-regulators. The Reichert type is the one in general use, although there are many features about the Dunham and Roux regulators which are advantageous.

If the pressure of the gas-supply varies from time to time, it is essential to regulate this by the use of a gas-pressure regulator (Murrill's is a cheap and satisfactory one).

Incubators, controlled electrically, can be obtained of certain foreign makers, and are quoted in catalogues of American dealers. It is probable that the Koch petroleum lamp incubator is the most satisfactory one where gas is not obtainable. They should be of all metal construction, and not with a wood casing, on account of the danger from fire. They cost from twenty-five to fifty dollars.

An incubator may be extemporized by putting the bulb of an incandescent electric lamp in a vessel of water. The proper temperature

may be obtained by increasing the amount of water or by covering the opening more or less completely with a towel. The test-tubes to be incubated can be put into a fruit jar or tin can, which receptacle is placed in the vessel heated by the lamp.

Emery suggests the use of a Thermos bottle as an incubator. As regards the matter of a low-temperature incubator (for gelatin work), this is best met by using a small refrigerator. The ice in the upper

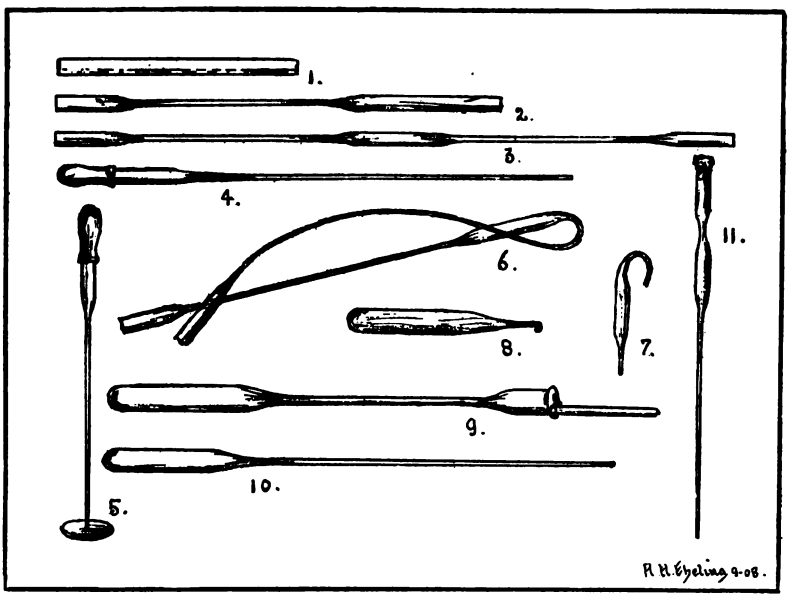

FIG. 6.—1, 2, 3, Drawing out glass tubing; 4, 5, Wright's rubber bulb capillary pipettes showing grease pencil mark for making dilutions; 6, 7, Wright's U tubes; 8, 9, 10, methods of drawing out test-tubes for vaccines in opsonic work; 11, bacteriological pipette.

part maintains an even cold, and by connecting up an electric lamp in the lower part of the refrigerator we can easily maintain a temperature which only varies one or two degrees during the twenty-four hours.

With a 16-candle-power lamp a temperature of about 25° C. is maintained (this is too high, being about the melting-point of gelatin); with an 8-candle-power, one about 21° to 23° C.; and with a 4-candle-power, from 18° to 20° C.; the box being about 20 x 30 x 36 inches.

When much serum reaction work is done, an electrically run centrifuge is of the greatest convenience.

A filter pump attached to the water faucet, preferably by screw threads, is almost indispensable for filtering cultures, etc., and for cleaning small pipettes, especially the hæmocytometer pipettes. Such a filter or vacuum pump with a vacuum gauge is more easily controlled.

With the possible exception of the platinum loop, there is no piece of apparatus so applicable to many uses as the capillary pipette made from a piece of glass tubing.

These may be made in a great variety of shapes. The one with a hooked end, the Wright tube, is the best apparatus for securing blood for serum tests. The crook hangs on the centrifuge guard and by filing and breaking the thicker part of the tube the serum is accessible to a capillary rubber bulb pipette or to the tip of a hæmocytometer pipette. In this way dilutions of serum are easily made. The capillary pipette is made by taking a piece of 1/4-in. soft German glass tubing, about 6 inches long, and heating in the middle in a Bunsen flame, revolving the tubing while heating it. When it becomes soft in the center, remove from the flame and with a steady even pull separate the two ends. The capillary portion should be from 18 to 20 inches in length. When cool, file and break off this capillary portion in the middle. We then have two capillary pipettes. By using a rubber bulb, such as comes on medicine droppers, we have a means of sucking up and forcing out fluids by pressure with the thumb and forefinger of the right hand. The bulb should be pushed on about 1/2 to 3/4 in.; this gives a firmer surface to control the pressure on the bulb.

A bacteriological pipette is made by drawing out a 9-inch piece of tubing about 3 inches at either end, then heating in the middle we draw out and have two pipettes similar to the one shown in the drawing. A piece of cotton is loosely pushed in just above the narrow portion. These may be wrapped in paper and sterilized for future use. They may be made perfectly sterile at the time of drawing out.

Where gas is not at hand, the Barthel alcohol lamp gives a flame similar to that of the Bunsen lamp and is equally satisfactory for heating glass tubing. By making a collar with a lateral opening to fit the burner of a Primus lamp a powerful side-flame is obtained which is almost as suitable for glass blowing as the Bunsen blast usually employed.

CHAPTER II.

CULTURE MEDIA.

WHILE there are certain advantages in sterilizing the glass test-tubes prior to filling them with media, yet this may be dispensed with—the sterilization after the media has been tubed being sufficient. If a dressing sterilizer is at hand, this is preferable for sterilizing such media as bouillon, potato, and agar (10 to 15 pounds' pressure for fifteen minutes). Milk should be sterilized with the Arnold, subjecting the media to three steamings for twenty minutes on three successive days. Gelatin may be sterilized in either way, but preferably in the autoclave at 7 pounds' pressure for fifteen minutes. As soon as taken out of the sterilizer it should be cooled as quickly as possible in cold water. This procedure tends to prevent the lowering of the melting-point of the finished gelatin and also preserves its spissitude.

Blood-serum is preferably solidified as slants in a blood serum inspissator. This requires one to two hours. The subsequent sterilization in the autoclave or Arnold should not be done immediately after making the solidified slants, but on the subsequent day. If done on the same day, many of the slants are ruined by being disrupted by bubbles. The preparation of blood-serum slants or slants of egg media can be conveniently carried out in a rice cooker (double boiler). Place the tubes in the inner compartment of the cooker, obtaining the slant desired by manipulating an empty test-tube, or with a towel or cotton batting on the bottom. Then cover the tubes with another towel. The outer compartment should contain water alone (no 25% salt solution). The inner compartment should be weighted down so that it is surrounded by water—the light tubes not being sufficient to sink it. Allowing the water in the outer compartment to boil one or two hours will inspissate or solidify the slants satisfactorily. The sterilization on subsequent days may be carried out in the same apparatus, although it is more efficient if done in an Arnold or an autoclave. (This sterilization in the rice cooker makes the media too dry.)

In making media a rice cooker is almost essential; at any rate, it is so if ease, expedition, and unfailing success in preparation are to be achieved. As it is necessary to make the contents of the inner compartment boil, the temperature of the water in the outer compartment must be raised. This is done by using a 25% solution of common salt or a 20% solution of calcium chloride in the outer compartment instead of plain water.

A 15% solution of salt raises the boiling-point 2 1/2° C.; a 20%, 3 1/2° C., and a 25%, 4 1/2° C. The raising of the boiling-point by calcium chloride is about the same for similar strength solutions.

Although the Bacteriological Committee of the A. P. H. Association recommends special steps to be taken in the preparation of gelatin and agar, yet for clinical purposes it will be found satisfactory to keep on hand a stock of buillon, and when it is desired to make agar or gelatin to simply prepare such media from the stock bouillon in the way to be subsequently given.

Nutrient Bouillon.

This may be made either from fresh meat or from meat extract. Media from fresh meat are usually lighter in color and possibly clearer. In the Philippines, however, certain measures employed for the preservation of the meat made it very difficult to prepare clear bouillon from it, so that meat extract was used entirely. There is very little difference, if any, in the nutritive power of media made in either way. The chief objections to fresh meat as a base are: 1. It takes more time and trouble. 2. The reaction, due to sarcolactic acid and acid salts, is quite acid, so that it is necessary to titrate and neutralize the excess of acidity. 3. The reaction of the finished media tends to change unless the boiling at the time of making was very prolonged. 4. It is not infrequent to have a heavy precipitate of phosphates thrown down at the time of sterilization, thus making it necessary to repeat the process of filtration and sterilization.

If fresh meat is used, take about 500 grams (one pound), remove fat and cut it up with a sausage mill or purchase the meat already cut up as for a Hamburg steak. It makes little difference whether the amount be 100 grams more or less. Place the chopped-up meat in a receptacle and pour 1000 c.c. of water over it. Keep in the ice chest over night

and the next morning skim off with a piece of absorbent cotton the scum of fat; then squeeze out the infusion with a strong muslin cloth, making the amount up to 1000 c.c. This meat infusion contains all the albuminous material necessary for the clarification of the bouillon. It is convenient to designate this meat base as Meat Infusion to distinguish from the base containing meat extract.

Having obtained 1000 c.c. of this 50% meat infusion, we dissolve in it 1% of Witte's peptone and 1/2% of sodium chloride. While there is a sufficiency of the various salts necessary for bacterial development in the meat juices, yet there is not enough to give the best results when bouillon cultures of various organisms are used for agglutination tests; and furthermore, when bouillon is used for blood cultures, disintegration of the red cells, with clouding of the clear medium, may occur if there be not sufficient salt present to prevent this.

The salt and the peptone are best put in a mortar, and adding about one ounce of the meat infusion we make a pasty mass; then we gradually add the remaining infusion until solution is complete. It is sometimes recommended to use a temperature of 50° C. to facilitate the solution of the peptone. This is not necessary, and if the temperature is not watched closely it might go up to 65° C. or higher and we should lose the clearing albuminous material from its coagulation. Of this rather cloudy solution take up 10 c.c. with a pipette and let it run out into a porcelain dish. Add 40 c.c. of distilled or rain water and about six drops of a 0.5% phenolphthalein solution. (Phenolphthalein, 0.5; dilute alcohol, 100 c.c.) Bring the contents of the porcelain dish to a boil and continue boiling for one or two minutes in order to expel all CO_2. Now from a burette filled with decinormal sodium hydrate solution, run in this solution until we have the development of a faint but distinct pink in the boiling diluted bouillon which is not dissipated on further boiling. Having obtained the light pink coloration we read off the number of c.c. or fractions of a c.c. of N/10 sodium hydrate solution added to produce the color. This number gives the acidity of the bouillon in percentage of N/1 acid solution.

Percent acid means that so many c.c. of N/1 acid added to 100 c.c. of the medium at the neutral point would give that percentage reaction. Thus 1 1/2 c.c. of N/1 HCl solution added to 100 c.c. of medium at 0, would give us 1 1/2% of acidity or +1.5.

Percent alkaline means so many c.c. of N/1 sodium hydrate solution

added to 100 c.c. of the medium at the neutral point. Thus a 1/2% alkaline medium would be one whose alkalinity would correspond to the addition of 1/2 c.c. of N/1 NaOH to 100 c.c. of the medium at 0. It is written −.5.

If we took 100 c.c. of the medium and put it in a beaker and then ran in N/1 NaOH solution from a burette, it will be readily understood that if we had to add 3 1/2 c.c. of N/1 NaOH to obtain the pink color, it would show that the acidity of the 100 c.c. of medium, being tested, corresponded to 3.5 c.c. of N/1 acid solution, and that its acidity was equal to 3 1/2% of N/1 acid solution, or that its reaction was +3.5.

As N/1 NaOH solution is too corrosive for general use in a burette, and as 10 c.c. of medium is more convenient to work with than 100 c.c., we use a solution one-tenth the strength of the N/1 NaOH and we take only one-tenth of the 100 c.c. of medium. In this way it is the same from a stand-point of directly reading off our percentage reaction as if we had 100 c.c. of medium and used N/1 NaOH solution. The A. P. H. Association recommends 5 c.c. of the medium and the use of N/20 NaOH. As the N/10 NaOH is always at hand for titrating gastric juice, the N/10 is used instead.

Should it be found difficult to carry on the titration while boiling the end reaction may be fairly accuately determined in the cold. Deliver into a beaker from a pipette 10 c.c. of the bouillon and make up to 50 c.c. with distilled water and add 5 drops of .5% phenolphthalein solution. Then run in N/10 NaOH from a burette and continue to add the N/10 NaOH solution from the burette, drop by drop, until the addition of a drop fails to show any intensifying of the purplish violet color at the spot where it came in contact with the diluted bouillon in the beaker. This marks the end reaction. A reaction of about +.7 in the cold gives a delicate pink with phenolphthalein as an indicator. Titration in the cold is not very satisfactory with gelatin and agar.

Having determined the percentage acidity of the 10 c.c. sample tested, we easily calculate the number of c.c. of N/1 NaOH solution required to be added to the 1000 c.c. of bouillon to obtain a reaction corresponding to the neutral point of phenolphthalein. It is more exact to take the average of two titrations.

As 100 c.c. of medium would require 3 1/2 c.c., 1000 c.c. would require 10 times as much, or 35 c.c. N/1 NaOH solution. Having measured out and added 35 c.c. of the N/1 NaOH solution to the meat

infusion, containing salt and peptone, we have a solution which is exactly neutral to phenolphthalein, or 0. It is usually considered that a reaction of about 1% acid is the optimum reaction for bacterial growth. Hence we should now add 1% of N/1 HCl solution to the medium. This would be accomplished by adding 10 c.c. of N/1 HCl solution to the 1000 c.c. of neutralized medium, and we would have a medium with a reaction of +1. If we desired a reaction of 1% alkalinity we would add an additional c.c. of N/1 NaOH solution to every 100 c.c. of the medium at 0, or 10 c.c. for the 1000 c.c. of medium. The reaction would then be −1.

As a matter of convenience, we usually determine the reaction of the medium, which is always more or less acid, and then add enough N/1 NaOH to reduce the acidity to the percentage we desire to set the medium, instead of neutralizing all the acidity present and then, in a second operation, restoring the acidity to the point desired.

Thus finding the acidity of the medium to be 3 1/2% and desiring to give it an acidity of 1%, we would add only 2 1/2 c.c. of N/1 NaOH to every 100 c.c. of medium, or 25 c.c. for the 1000 c.c. of medium. The reaction would then be found to be +1.

The neutral point of litmus is not a sharp one, but it corresponds rather closely with a reaction of +1.5 to phenolphthalein. The recommendations of the A. P. H. Association call for making the titration with the medium boiling. If the color of the end reaction at boiling-point be obtained, it will be found that when cool it deepens until it corresponds to the rich violet-pink of the end reaction in the cold or *vice versa*.

To summarize:

Take Peptone,	10 grams
Sodium chloride,	5 grams
50% meat infusion,	1000 c.c.

Dissolve the peptone and sodium chloride in the meat infusion and add enough N/1 NaOH to make the reaction +1.

Put the solution in the inner compartment of a rice cooker and bring to the boiling-point and maintain this temperature for twenty minutes. The calcium chloride or sodium chloride in the outer compartment of the rice cooker enables us to secure a boiling temperature for the contents of the inner compartment. Do not stir the bouillon that is being

heated, as the pultaceous membranous mass of coagulated albumin makes filtration easy. Filter. The filter-paper in the funnel should be thoroughly wet with water before pouring on the bouillon. This is to prevent clogging of the pores of the filter-paper. Make up the quantity of filtrate to 1000 c.c. by adding water.

If greater exactness is demanded than answers for ordinary clinical work, it is advisable to again titrate and again adjust the reaction or to simply record the exact reaction. It is more convenient to have a counterpoise to balance the inner compartment and then to add water to the medium until a kilo weight, in addition to the weight balancing the container, is just balanced. Then titrate, adjust the reaction (if so desired), and filter. Sterilize in the autoclave at 115° to 120° C. for fifteen minutes or in the Arnold on three successive days. The use of a balance is preferable in the preparation of bouillon, necessary in making gelatin and imperative in making agar media.

Bouillon Made from Liebig's Meat Extract.

Place in a mortar 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride. Dissolve the whites of one or two eggs in 1000 c.c. of water. Then add this egg-white water, little by little, to the extract, peptone, and salt in the mortar until a brownish solution is obtained. Pour this into the inner compartment of a rice cooker; apply heat to the outer compartment containing the salt or calcium chloride solution, allow to come to a boil and to continue boiling for fifteen to twenty minutes. Do not stir. Place inner compartment on the scales and its counterpoise and a one-kilo weight on the other side. Add water until the two arms balance. Filter and sterilize.

The reaction of media made with Liebig's meat extract rarely exceeds +.75 (from +.6 to +.9). Consequently for growing bacteria it is unnecessary to titrate and adjust reaction unless precision is demanded.

Sugar-free Bouillon.

Inoculate nutrient bouillon in a flask with the colon bacillus. Allow to incubate at 37° C. over night. Pour the contents into a sauce-pan and bring to a boil to kill the colon bacilli. Put about 15 grams of purified talc (Talcum purificatum, U. S. P.) in a mortar. Add the dead

colon culture, stirring constantly. Then filter through filter-paper. It may be necessary to again pass the filtrate through the same filter until the sugar-free bouillon is perfectly clear.

For all ordinary purposes the very small amount of sugar in bouillon made from Liebig's meat extract may be neglected in determining gas production; so that under such conditions the various sugars could be added directly to the meat-extract bouillon.

Sugar Bouillons.

The sugar media ordinarily used for determining fermentation or gas production are those of glucose and lactose. In special work such carbohydrates as saccharose and maltose are used. The alcohol mannite is used in differentiating stains of dysentery bacilli.

To make, simply dissolve 1 or 2% of the sugar in sugar-free bouillon or that made from meat extract. Tube in Durham's or the ordinary fermentation tubes and sterilize in the autoclave at only about 5 pounds' pressure for 15 minutes, or in the Arnold.

Too high a degree of heat may turn the sugar bouillon brownish. The nature of the sugar itself may further be affected by too high a temperature.

Glycerine Bouillon.

Add 6% of glycerine to ordinary bouillon. It is chiefly used in the cultivation of tubercle bacilli.

Peptone Solution (Dunham's).

Dissolve 1% of Witte's peptone and 1/2% of sodium chloride in distilled water. Filter, tube, and sterilize. Peptone solution may be used as a base for sugar media instead of bouillon. It is the medium used in testing for indol production. This test is made by adding from 6 to 8 drops of concentrated H_2SO_4 to a twenty-four- to forty-eight-hour-old peptone culture of the organism to be tested. If the organism produces both indol and a nitroso body, we obtain a violet-pink coloration, "cholera red." If no pink color is produced on the addition of the sulphuric acid, add about 1 c.c. of an exceedingly dilute solution (1 : 10,000) of sodium nitrate.

It is very important in determining the "cholera red" reaction to know that the peptone used will give the reaction as it is not given by true cholera stains with certain samples of peptone.

Nutrient Agar.

In making agar medium it is preferable to use powdered agar, as this goes into solution more readily than the shredded agar. The reaction of agar is slightly alkaline, so that if 1 1/2 to 2% of agar is added to nutrient bouillon having a reaction of +1 the finished product will be found to be about +.8.

To make: Weigh out 15 to 20 grams of powdered agar and place in a mortar. Make a paste by adding nutrient bouillon, little by little, and when a smooth even mixture is made, pour it into the inner compartment of a rice cooker and add the remainder of the 1000 c.c. of bouillon. The use of the balance is preferable.

The outer compartment of the rice cooker should contain the 25% salt solution. Bring to boil, and the agar will be found to have entirely gone into solution after five to ten minutes of boiling.

Then, using a funnel which has been heated in boiling water and which contains a small pledget of absorbent cotton, we filter the agar, tube it, and sterilize it in the autoclave or Arnold. One and one-half percent agar can be readily filtered through filter-paper and gives a clearer medium.

By taking of meat extract 3 grams, peptone 10 grams, salt 5 grams, powdered agar 15 grams, the white of one egg and 1000 c.c. of water, making at first a paste of all the ingredients in a mortar, then gradually adding the remainder of the 1000 c.c. of water, putting in the rice cooker, bringing to a boil without stirring, allowing to boil fifteen minutes and then filtering through absorbent cotton in a hot funnel, we obtain a satisfactory medium, the reaction of which will be from +.7 to +.9.

Glucose Agar.

Add the agar to 1 or 2% glucose bouillon and proceed as for ordinary agar.

Glycerine Agar.

Add the agar to 6% glycerine bouillon instead of nutrient bouillon.

Glycerine Agar Egg Medium.

Take the white and the yolk of one egg and mix thoroughly in a vessel kept between 45° and 55° C. with an equal amount of glycerine agar. Tube the medium, inspissate in a rice cooker as for serum tubes, and sterilize as for blood-serum tubes.

This makes an excellent medium for growing tubercle bacilli. As egg medium has a tendency to be dry, it is well to add 1 c.c. of glycerine bouillon to each slant before autoclaving.

Nutrient Gelatin.

Add about 12% (120 grams) of "gold label" gelatin to 1000 c.c. of nutrient bouillon in a rice cooker. If the bouillon had a reaction of about +1, the gelatin solution will be about +3.5, so that it is always necessary to titrate gelatin and neutralize to about +1. The procedure is the same as for bouillon. As the color reaction is not quite as distinct with gelatin, it is better to make a color standard with 4 or 5 c.c. of the gelatin medium in 50 c.c. of water, instead of using the distilled water alone, as was recommended for bouillon.

Having neutralized and allowed to boil for fifteen minutes, we filter through filter-paper in a hot funnel. As it is very important that gelatin should be perfectly clear, it is better to filter through filter-paper than through cotton. The filter-paper should be very thoroughly wetted with very hot water before filtering gelatin or agar.

Tube the medium and sterilize, either in the Arnold on three successive days or in the autoclave at 8–10 pounds' pressure for ten minutes. The tubes should be cooled as quickly as possible in cold water after taking out of the sterilizer.

Litmus Milk.

Milk for media should be as fresh as possible. It should then be put in a 1000 c.c. Erlenmeyer flask, sterilized for fifteen minutes in the Arnold, and set over night in the refrigerator. The next morning the milk beneath the cream should be siphoned off. The short arm of the siphon should not reach the bottom of the flask so as to avoid the sediment. Add sufficient litmus solution to this milk to give a decided lilac tinge; tube and sterilize in the Arnold on three successive days.

Litmus milk which apparently is as satisfactory as the above as regards nutritive quality and cultural characteristics can be made from certain canned milks which have not been condensed or sweetened and which do not contain chemical preservatives. The "Natura" brand of milk is the one I have experimented with.

Litmus Solution.—A simple solution may be made by digesting the powdered cubes repeatedly with hot water, mixing the extracts, and, after allowing them to stand all night, decanting the solution from the inert sediment into a clean bottle.

In litmus solution so made, however, a red dye is also present while calcium and other salts are dissolved out. For bacteriological purposes a pure solution of the blue dye should be used. This is called "azolitmin." It is freely soluble in water but insoluble in alcohol.

It can be conveniently prepared as follows: Weigh out 2 ounces of powdered litmus; digest repeatedly with fresh quantities of hot water until all the coloring matter is dissolved out; allow to settle, and decant off the fluid from the insoluble powder. Add together the extracts, which should measure about a liter. Evaporate down the solution to a moderate bulk, then add a slight excess of acetic acid, so as to convert all carbonates present into acetates. Continue the evaporation, the later stages over a water bath, until the solution becomes pasty. Add 200 c.c. of alcohol, and mix thoroughly. The alcohol precipitates the blue coloring matter, while a red coloring matter, together with the alkaline acetate present, remains in solution. Transfer to a filter. Wash out the dish with alcohol and add this to the filter. Wash the precipitate on the filter with alcohol. Dissolve the pure coloring matter remaining on the filter in warm distilled water and dilute to 500 c.c. Azolitmin solution prepared in this way is more sensitive than ordinary litmus solution.

Azolitmin in powder can be purchased from dealers in chemicals.

Potato Slants.

Take Irish potatoes and scrub thoroughly with a stiff brush. Then pare off generously all the outer portion. From the white interior cut out cylinders with a cork borer. These cylinders should be of 1/2 to 3/4 of an inch in diameter. Divide a cylinder by a diagonal cut. This gives a plug with a flat base, the other extremity being a slant. These

potato plugs should be left in running water over night or washed with frequent changes of water. This prevents the blackening of the plug. Into a 1-in. test-tube drop a pledget of absorbent cotton well moistened with water. Then drop in the potato plug, base downward. Sterilize in the autoclave at 15 pounds for fifteen to twenty minutes, to insure sterility.

FIG. 7.—Potato in culture tube. (*Williams.*)

For glycerine potato, soak the plugs in 6% glycerine solution for about one hour. Then drop a pledget of absorbent cotton moistened with the same glycerine solution into the test-tubes and follow it with the potato plug. Sterilize in the autoclave.

BLOOD-SERUM.

The blood of cattle should be collected in large pans or pails at the abattoir. This vessel of blood should then be kept in the cold-storage room and the next morning the more or less clear serum will have been squeezed out from the clot. Collect this serum and keep in the ice chest for future use. If to be kept for a long time, it is advisable to add about 2% of chloroform to the serum in tightly corked flasks. This will not only keep the serum, but will eventually sterilize it.

To make Löffler's serum, take one part of glucose bouillon and three parts of blood-serum. Mix, tube, and coagulate the albumin in the inspissator or rice cooker, giving the tubes a proper slant before heating. Sterilize the following day in the autoclave as previously directed or in the Arnold on three successive days.

A SUBSTITUTE FOR ORDINARY BLOOD-SERUM.

Add from 10 to 15 c.c. of 1% glucose bouillon to the white and yolk of one egg, make a smooth mixture in a mortar and tube. Inspissate and sterilize as for ordinary serum slants. The morphology of the diphtheria bacilli and the luxuriance of growth is similar to that of cultures on Löffler's serum.

This is better than the various white of egg substitutes usually recommended.

Hydrocele, and Blood Agar.

To tubes of melted agar at 50° C. add from 1 to 3 c.c. of hydrocele or ascitic fluid, observing aseptic precautions. For blood agar the blood from a vein should be received into a sodium citrate salt solution to prevent coagulation, and added subsequently as for hydrocele fluid. Allow the agar to solidify as a slant.

Blood-streaked Agar.

Sterilize the lobe of the ear and puncture with a sterile needle. Collect the exuding blood on a large platinum loop and smear it over the surface of an agar slant. It is advisable to incubate over night as a test for sterility.

Bile Media.

Secure ox bile from the abattoir or human bile from cases of gall-bladder drainage in hospitals. Put about 10 c.c. in each tube and sterilize. Some prefer to add 1% of peptone.

This is the medium for blood cultures in typhoid, etc.

The bile lactose medium now used in water analysis is made by adding 1% of lactose to ox bile and tubing in fermentation tubes. As a substitute for fresh bile one may use a 15 to 20% solution of a good quality of inspissated ox gall (Fel Bovis Purificatum).

Plating Media for Fæces Work.

The media of Endo, Conradi-Drigalski and the lactose litmus agar medium are probably the most satisfactory of the numerous ones that have been proposed for plating out fæces. A convenient way of preparing any one or all of these, and which apparently gives media equal to that prepared according to the original formulæ, is as follows:

Liebig's extract,	5 grams.
Salt,	5 grams.
Peptone,	10 grams.
Lactose (C. P.),	10 grams.
Agar,	30 grams.
Water to make	1000 c.c.

Prepare as for ordinary nutrient agar, with the difference that the reaction should be brought down to −.5.

A stiff agar (3%) is employed to check the diffusion of acid beyond the colony.

For Endo's Medium.—Keep this lactose agar base in 100 c.c. quantities in Erlenmeyer flasks instead of test-tubes.

When needed for plating, melt a flask of this agar, and while liquid add to the 100 c.c. ten drops of a saturated alcoholic solution of basic fuchsin, and then twenty drops of a freshly prepared 20% solution of sodium sulphite. If only occasionally using such media, tube in 10 c.c. quantities and add one drop of the basic fuchsin and 2 drops of the sodium sulphite solution to a tube of the melted lactose agar base just before pouring the plate. The medium should be of a light flesh or pale salmon color.

Colon bacilli show on this medium as vermilion colonies, which in about forty-eight hours have a metallic scum on them. Typhoid and dysentery colonies are grayish.

For Lactose Litmus Agar.—Color the lactose agar base with tincture of litmus to a lilac color. This may be tubed, putting 10 c.c. in each test-tube, or put in quantities of 50 or 100 c.c. in small Erlenmeyer flasks. It is then sterilized in the autoclave (10 pounds for fifteen minutes) or in the Arnold.

For Conradi-Drigalski Medium.—Melt down the lactose litmus agar in the flasks holding 100 c.c. Then add to the 100 c.c. of medium 1 c.c. of a solution of crystal violet (crystal violet 0.1 gram, distilled water 100 c.c.). The medium is then ready to put into plates. Colon colonies are pink. Typhoid and dysentery colonies, a bluish-gray.

Conradi's Brilliant Green Medium.—Take of Liebig's extract 20 grams (2%), peptone 10 grams (1%), agar 30 grams (3%) and water to 1000 c.c. This amount of meat extract should give about the proper acidity, +3. If not, the reaction should be adjusted to that point. Filter through cotton, tube 150 c.c. amounts into 250 c.c. Erlenmeyer flasks and sterilize.

Then add 1 c.c. of a 1 to 1000 aqueous solution of brilliant green (Höchst) and 1 c.c. of a 1% solution of picric acid to the flasks containing 150 c.c. of the melted agar. Sterilization after adding the dyes precipitates them and is unnecessary. Pour the finished medium into large Petri dishes and inoculate the surface with the fæces.

Brilliant green does not interfere with agglutination as does malachite green.

This medium is the one of choice in isolating typhoid bacilli from fæces and urine.

The surface of the poured plates of Endo, Conradi-Drigalski, and the brilliant green media should be dried in the incubator before smearing with the fæces.

Culture Media for Protozoa.

Medium of Musgrave and Clegg.—Dissolve in 1000 c.c. of water 0.3 to 0.5 gram Liebig's extract and 0.3 to 0.5 gram of common salt. If desired for plating add 2 to 3% of agar.

A very satisfactory substitute is ordinary nutrient bouillon diluted one to ten.

Medium of Smith.—Glucose 1.0 gram; Peptone 1.0 gram; NaCl 0.2; Aqua destill. 1000.0; Na_2CO_3 0.3. Agar q. s. is added for solid medium.

Medium of Castellani.—This is an aqueous medium containing 1% of lactose and 10% of egg albumin. This may replace water of condensation in an agar slant.

CHAPTER III.

STAINING METHODS.

In order to study a bacterial or blood specimen the first essential is a properly prepared film; the matter of staining is of less importance. The slide or cover-glass, after cleaning with soap and water or by special solutions, should be polished with a piece of old linen. If a glass surface is free of grease a loopful of water will smear out evenly and over the entire surface. The only quick practical way to make the slide or cover-glass grease free is to burn the surface for a moment in a Bunsen or alcohol flame. The cover-glass must not be warped. To make a preparation, apply a small loopful of distilled water on the slide or cover-glass and, touching a colony with a platinum needle, stir the transferred culture into the loopful (not drop) of water. The mistake is almost invariably made of taking up too much bacterial growth. Fluid cultures do not need dilution. Smearing the mixture over a large part of the cover-glass or over an equal area of a slide, it is allowed to dry. If very little water is used, the preparation dries readily. Otherwise it can be dried in the fingers high over a flame. As soon as dry, the cover-glass should be passed three times through the flame, film side up, to fix the preparation. Slides may be fixed by passing them five times through the flame, but the method by burning alcohol recommended for fixing blood-films gives more satisfactory bacterial fixation. For routine work the stain recommended is a dilute carbol fuchsin. Drop about 5 to 10 drops of water on the cover-glass, then add one drop of carbol fuchsin. Allow the dilute stain to act from one to two minutes, then wash in water, dry between small squares of filter-paper (4 x 4 inches), and mount in balsam or the oil used for the 1/12-inch immersion objective. Some prefer to mount directly in water without preliminary drying. It is good practice to make a rule to always keep the smeared side of the preparations up—never allowing it to be reversed. By this simple rule, preparations can be carried through the most complicated staining methods without the necessity of scratching the cover-glass, etc., to see which is the film side. In grasping a cover-glass with a Cornet or Stewart

forceps, be sure that the tips are well by the margin of the glass, otherwise the stain will drain off. In staining with slides, the grease pencil and the glass tubing, as recommended under Blood Smears, will be found useful. The dilute carbol fuchsin and Löffler's methylene blue are probably the best routine stains.

Löffler's Alkaline Methylene Blue.—Saturated alcoholic solution of methylene blue, 30 c.c.; one to ten thousand caustic potash solution, 100 c.c. (Two drops of a 10% solution KOH in 100 c.c. of water makes a 1 : 10,000 solution.)

Carbol Fuchsin (Ziehl-Neelsen).—Saturated alcoholic solution basic fuchsin, 10 c.c.; 5% aqueous solution carbolic acid, 100 c.c.

Gram's Method.—The most important staining method in bacteriological technic and the one so rarely giving satisfactory results to the inexperienced is Gram's stain. In using this method, the following points must be kept in mind:

1. Laboratory cultures (subcultures) which have been carried over for years frequently lose their Gram characteristics.

2. Cultures which are several days old or dead or degenerated do not stain characteristically.

3. The aniline gentian violet deteriorates when exposed to light in two or three days—it should be kept in the dark. It should have a rich, creamy, violet appearance.

4. The iodine solution deteriorates and becomes light in color. It should be of a rich port-wine color.

5. The decolorizing with 95% alcohol should stop as soon as no more violet stain streams out. This is best observed over a white background, washing at intervals. Do not confuse stain on forceps for that on preparation.

6. The preparation should be thin and evenly spread. Some prefer carbol gentian violet to aniline gentian violet. (Saturated alcoholic solution of gentian violet, 1 part; 5% aqueous solution of carbolic acid, 10 parts.) This tends to overstain.

The formula for aniline gentian violet is 1 part of saturated alcoholic solution gentian violet and 3 parts of aniline oil water (made by adding 2 c.c. aniline oil to 100 c.c. distilled water, shaking violently for three to five minutes and then filtering several times, to get rid of the objectionable oil droplets which, in a Gram-stained preparation, show as confusing black dots).

An easily made and quite permanent gentian violet stain is the following:

Saturated alcoholic solution gentian violet in	25 c.c.
Solution of formaline (5 %),	75 c.c.

The following stock solutions of Weigert are recommended:

No. 1.		No. 2.	
Gentian violet,	2 grams.	Gentian violet,	2 grams.
Aniline oil,	9 c.c.	Distilled water,	100 c.c.
Alcohol (95%),	33 c.c.		

These stock solutions keep indefinitely. Mix 1 c.c. of No. 1 with 9 c.c. of No. 2. Filter. This keeps about two weeks and is the solution to pour on the preparation. It may be kept on from two to five minutes. Some hasten the staining by steaming as for tubercle bacilli. Next wash the preparation with water and flood the cover-glass with Gram's iodine solution. Some bacteriologists simply pour off excess of aniline gentian violet and immediately drop on the iodine solution. It is well to repeat the application of the iodine solution a second time. The iodine solution is left on one minute or until the preparation has a coffee-grounds color.

Gram's Iodine Solution.

Iodine,	1 gram.
Potassium iodide,	2 grams.
Distilled water,	300 c.c.

After washing off the excess of iodine solution at the tap, drop on 95% alcohol and decolorize until no more violet color streams out. Now wash again and counterstain either with the dilute carbol fuchsin or with a saturated aqueous solution of Bismarck brown.

The Gram-positive bacteria are stained a deep violet.

In staining smears of pus for gonococci or other Gram-negative bacteria it is best to first stain with the gentian-violet solution for two to five minutes. Then wash and examine the preparation mounted in water. The organisms stand out prominently. After noting the presence of the cocci treat the smear with the Gram solution and proceed as in the usual Gram staining technic.

Stained by Gram's Method.	*Not Stained by Gram's Method.*
S. pyogenes aureus.	Meningococcus.
S. pyogenes albus.	M. catarrhalis.
S. pyogenes.	M. melitensis.
M. tetragenus.	B. typhosus.
Pneumococcus.	B. coli communis.
Anthrax bacillus.	B. dysenteriæ (Shiga).
Tubercle bacillus.	Sp. choleræ asiaticæ.
Lepra bacillus.	B. pyocyaneus.
Tetanus bacillus.	B. mallei.
Diphtheria bacillus.	B. pneumoniæ (Friedländer).
B. aerogenes capsulatus.	B. proteus.
Oidium albicans.	B. of influenza.
Mycelium of actinomyces.	B. of bubonic plague.
Saccharomyces.	B. of chancroid.
Hofman's bacillus.	B. of Koch-Weeks.
B. xerosis.	Gonococcus.

Practically all pathologic cocci are Gram-positive, except the Gonococcus, the Meningococcus, the M. catarrhalis, and the M. melitensis.

Practically all pathogenic bacilli are Gram-negative, except the spore-bearing ones (exception B. malig. œdemat.), the acid-fast ones and diphtheria and diphtheroid organisms.

Method for Staining Acid-fast Bacilli.—1. Carbol fuchsin, with gentle steaming for one to two minutes or in the cold three to five minutes.

2. Wash in water.

3. Decolorize in 95% alcohol containing 3% of hydrochloric acid (acid alcohol), until only a suggestion of pink remains—almost white.

4. Wash in water.

5. Counterstain in saturated aqueous solution of methylene blue or with Löffler's methylene blue.

6. Wash, dry, and mount.

Smith's formol fuchsin.

Saturated alcoholic solution basic fuchsin,	10 c.c.
Methyl alcohol,	10 c.c.
Formalin,	10 c.c.
Distilled water to make	100 c.c.

This gives a very sharp differentiation of bacteria and nuclear structures. It has a purplish tinge. Fixation by heat gives the best staining. Allow the stain to act for two to ten minutes. It should not be used until after standing twenty-four hours, and after standing about two weeks it appears to lose its sharp staining power.

Archibald's Stain.—This is an excellent bacterial stain and has been highly recommended by Blue and McCoy in plague work.

Solution No. 1.		*Solution No. 2.*	
Thionin,	.5	Methylene blue,	.5
Phenol crys.,	2.5	Phenol crys.,	2.5
Formalin,	1.0	Formalin,	1.0
Water,	100.0	Water,	100.0

Dissolve for twenty-four hours. Mix equal parts and filter. Stains smears fixed by heat or otherwise in ten seconds.

Romanowsky Stains.—See under section on Blood.

Neisser's Stain for Diphtheria Bacilli.

Solution No. 1.		*Solution No. 2.*	
Methylene blue,	0.1 gram.	Bismarck brown,	0.2
Alcohol,	2 c.c.	Water (boiling),	100 c.c
Glacial acetic acid,	5 c.c.	Dissolve the stain in the boiling water and filter.	
Distilled water,	95 c.c.		

Dissolve the methylene blue in the alcohol and add it to the acetic acid water mixture. Filter.

To stain: Fix the preparation. Pour on the dilute acetic acid methylene blue solution and allow to act from thirty to sixty seconds. Wash. Then pour on the Bismarck-brown solution, and after thirty seconds wash off with water. Dry and mount. The bodies of the bacilli are brown with dark blue dots at either end.

Neisser recommends only five seconds as the time of application of each solution. He also recommends that the culture be only nine to eighteen hours old and that the temperature of the incubator do not exceed 36° C. Incubation at 37° C. gives satisfactory results.

Capsule Staining.—The best method for studying bacteria, as to presence of capsules, is in the hanging drop, with the greater part of the light shut off by the diaphragm.

In material where capsules are well developed, as in pneumonic sputum, the Gram method of staining brings out the capsule perfectly. This is of diagnostic value, as the more or less nonpathogenic pneumococci common about the mouth do not seem to show a capsule when stained in this way.

The most beautiful method of staining capsules is the latest one proposed by Muir.

1. Prepare thin film, dry and stain in carbol fuchsin one-half minute; the preparation being gently heated (steamed).
2. Wash slightly in 95% alcohol, then wash well afterward in water.
3. Flood preparation in mordant for five to ten seconds.

Mordant.—	Sat. aqueous sol. mercuric chloride,	2 parts
	Tannic acid (20% aqueous sol.),	2 parts
	Sat. aqueous sol. potash alum,	5 parts

4. Wash in water thoroughly.
5. Treat with 95% alcohol for one minute. (The preparation should have a pale red color.)
6. Wash well in water.
7. Counterstain with methylene blue one-half minute.
8. Dehydrate in alcohol. Clear in xylol and mount. (May simply dry specimen with filter-paper.)

Flagella Staining.—Inoculate a tube of sterile water (gently) in upper part, with just enough of an eighteen to twenty-four-hour-old agar culture to produce faint turbidity. Incubate for two hours at 37° C. From the upper part of culture take a loopful and deposit it on a cover-glass. Dry in thermostat for one to five hours or overnight. Use perfectly clean cover-glasses. To stain by

Muir's Modified Pitfield Method.—1. Flood specimen with mordant. Steam gently one minute.

Mordant.—	Tannic acid (10% aqueous solution),	10 c.c.
	Sat. aq. sol. mercuric chloride,	5 c.c.
	Sat. aq. sol. alum,	5 c.c.
	Carbol fuchsin,	5 c.c.

Allow precipitate to settle or centrifuge. Keeps only one week.

2. Wash well in water for two minutes.
3. Dry carefully—preferably in incubator.
4. Pour on stain. Steam gently one minute.

Stain.—Sat. aq. sol. alum, 10 c.c.
Sal. alc. sol. gentian violet, 2 c.c.
(May use carbol fuchsin instead of gentian violet.)
Stain only keeps two days.

5. Wash well in water. Dry and mount.

Spore Staining.—The most satisfactory spore staining method is really the negative staining of the spore obtained when a bacterial preparation is stained by dilute carbol fuchsin or Löffler's methylene blue. The spore appears as a highly refractile piece of glass in a colored frame.

The acid-fast method, as for tubercle bacilli, gives good results. The decolorizing, however, must be lightly done, otherwise the spore will lose its red stain.

The Staining of Protozoa.

Unless staining albuminous material it is well to add a little blood serum or white of egg to the preparation—about one loopful to a smear. The serum or white of egg is best preserved by the addition of 2% chloroform and kept tightly corked.

Giemsa's Method.—Fix moist smears with a fixative made by adding 1 part of 95% alcohol to 2 parts of saturated aqueous solution of bichloride of mercury. Keep in this solution twelve hours. Now wash for a few seconds in water and then for about five minutes with a dilute Lugol's solution (KI, 2 gm.; Lugol's solution, 3 c.c.; Aqua, 100 c.c.). Now wash in water and then in a .5% solution of sodium thiosulphate to remove the iodine which was used to remove the mercury. Wash in water five minutes, then stain with Giemsa's stain as used in blood work for one to ten hours. Wash and mount.

Vital Staining of Protozoa with Neutral Red Solution.—As a stock solution one uses a .5% aqueous solution of neutral red.

The drop of salt solution or water on the slide should be tinged a light violet-rose color with a fraction of a loopful and the fæces or other material emulsified in this.

Protozoa take a rose-pink color with a distinct differentiation between endoplasm and ectoplasm.

Should the fæces be quite alkaline the neutral red will be decomposed with the formation of bilirubin-like crystals.

The Giemsa formalin method described under Blood Work is of value in certain cases.

CHAPTER IV.

STUDY AND IDENTIFICATION OF BACTERIA—GENERAL CONSIDERATIONS.

In order to study bacteria it is necessary to isolate them in pure culture. This may be accomplished by taking one or more loopfuls of the material and mixing it in a tube of melted agar or gelatin. From this first tube one or more loopfuls are transferred to a second tube of melted agar or gelatin, and from this a third transfer is made, thereby giving us tubes in which the distribution of the bacteria is one or more hundred times less in the second than in the first tube, and equally more dilute in the third than in the second. When we pour the contents of the tubes into Petri dishes we would have the bacterial colonies on the first plate so thick that it would be impossible to pick up a single colony with a platinum needle without touching an adjacent one. On the second plate the distribution might be such that we should have discrete, well separated colonies, material from which could be taken up on the point of the needle or loop without touching any other colony. If the second plate did not meet these requirements, the third would.

In clinical bacteriology we work almost entirely with organisms preferring blood-heat temperature, hence it is necessary to use agar or blood-serum as standard media for the obtaining of isolated colonies. Gelatin is of little value for this purpose in medical work. In using agar it will be remembered that it solidifies at a temperature slightly below 40° C. and does not melt again until it is subjected to a temperature practically that of boiling. Again, if the temperature of the media exceeds 44° C. it may affect injuriously the organisms we wish to study. Consequently it requires careful attention and quick work to inoculate the tubes, mix, transfer and pour into plates within the limits of a temperature which injures the organisms, and one which brings about the solidification of the agar.

Again, we not only have colonies developing from organisms which have been fixed at the surface as the agar solidified in the plate, but more numerous ones developing from bacteria caught in the depths

of the media. Therefore we have superficial and deep colonies. Except to the person of great experience, all deep colonies look alike and there is at times great difficulty in deciding whether a colony is deep or superficial. It is in the matter of trying to obtain information from the differences

FIG. 8.—Petri agar plate. Made by spreading scrapings from the mouth over sterilized nutrient agar; after forty-eight hours in the thermostat the light "colonies" develop. Streaked plate. (*Delafield and Prudden.*)

in deep colonies that the greatest difficulties in the study of bacteriology arise. By using the method of simply stroking plates along five or six parallel lines from one side of the plate to the other with a glass rod, loop, or swab, we obtain colonies which are well separated and which are entirely superficial.

The material as pus, fæces, throat membrane, etc., should be evenly distributed in a tube of sterile water or bouillon; the swab which was originally used for obtaining the material being then pressed against the sides of the test-tube to express excess of fluid and then stroked gently over successive lines on one plate. Or, if the organisms be very abundant, over a second plate without recharging it from the inoculated tube.

To obtain isolated colonies on blood-serum or blood-streaked agar, which can be touched and by transfer obtained in pure culture, we simply smear the material on a slant of either medium. Then, without sterilizing the loop, we smear it thoroughly over a second slant, and so on to a third, or possibly a fourth or fifth.

At present the classification of the bacteria is very unsatisfactory from a scientific standpoint. The nomenclature abounds in instances where three or four terms are used in naming a single bacterium, instead of the single generic name and single specific one as is used in zoölogical nomenclature. This matter of nomenclature is a subordinate factor in the confusion when we begin to investigate and find that different names have been applied to apparently the same organism. The slightest variation in morphological, locomotor, or biological characteristics seems to be considered sufficient by many observers to justify the description of a new species, and, of course, the giving of a new name. Many of these names which are now retained were applied prior to the epoch-making introduction of gelatin media by Koch (1881) and consequently at a time when the isolation of organisms in pure culture was a matter of extreme difficulty and uncertainty. One of the first facts noted by the student in taking up bacteriology is the difficulty in determining motility; this property should always be tested on young cultures in bouillon. In Brownian movement there is a sort of scintillating movement, but the bacterium does not move from that part of the field. In current movement all the bacteria swarm in the same direction, going very fast at times, and then more slowly. If in great doubt, the mounting of the organisms in a 2% solution of carbolic acid will stop movement if it be true functional motility, while Brownian and current movement are not interfered with. In true motility bacteria move in opposite and in all directions, and move away from the place where first observed unless degenerated or dead.

Reaction of media is of the greatest importance in causing variation

in the functions of bacteria, and is one which has until recently been almost entirely neglected. In describing an organism at the present time it is always necessary to note the reaction of the media, the temperature at which cultivation took place, and the age of the culture when examined.

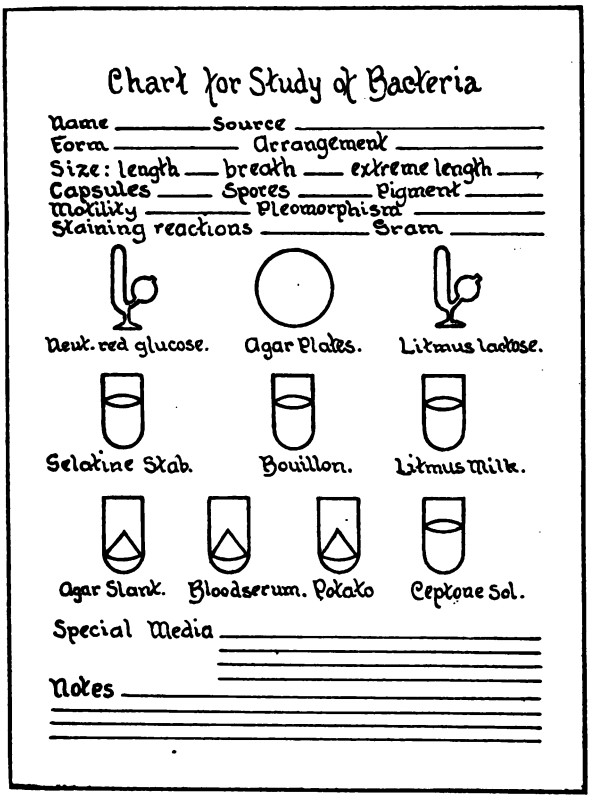

FIG. 9.—Chart in use at the U. S. Naval Medical School.

In the following keys the term bacterium has been used as a general designation for all schizomycetes. Migula calls motile rod-shaped organisms bacilli, and nonmotile ones bacteria. Lehmann and Neumann call spore-bearing organisms bacilli, and nonspore-bearing ones bacteria.

The B. typhosus is very motile and does not possess spores. According to Migula, it would be the Bacillus typhosus; according to Lehmann

and Neumann, the Bacterium typhosum. The B. anthracis has spores and is nonmotile. Hence it would be Bacterium anthracis, according to Migula, and the Bacillus anthracis, according to Lehmann and Neumann.

In the use of the keys at the head of each group of organisms it will be observed that the primary separation is on the basis of morphology —the cocci in one group, the bacilli in three subgroups: one for those rod-shaped organisms showing branching and curving forms, one for the spore bearers and one for the simple rods. The spirilla are grouped by themselves.

An important method of differentiation is the reaction to Gram's stain. It should be remembered that organisms carried along on artificial media often lose their Gram staining characteristics; hence it is desirable to determine this staining reaction in cultures freshly isolated. Be sure that the stains, especially the aniline gentian violet and the iodine solution, have not deteriorated. There is no more important stain than this, and none which requires greater experience. The chief causes of conflicting results are 1. working with old cultures and 2. not having satisfactory staining solutions.

Motility, as stated above, is at times difficult to determine. For this purpose young eighteen-hour-old bouillon cultures are preferable, and the preparation should be made by applying a vaseline ring to the slide, then putting a drop of the bouillon culture in the center of the ring (or a drop of water inoculated from an agar slant growth), then putting on a cover-glass. By this method current movement is done away with and the preparation keeps for hours. This is a convenient method for agglutination tests.

Liquefaction of gelatin is a very important means of differentiating. When a room-temperature incubator is not at hand (20° to 22° C.), it is better to put the inoculated gelatin tube in the body-temperature incubator, and from day to day test the power of solidifying with ice-water. If the organism digests the gelatin (a liquefier), the medium will remain fluid when placed in ice-water; if the organism is a nonliquefier, the medium in the tube becomes solid. Of course we lose the information to be obtained from the shape of the area of liquefaction.

For routine work the only sugar media used are the glucose and the lactose bouillon. These are of the utmost importance in differentiating organisms of the typhoid and colon group. Following Ford, these

intestinal bacteria have primarily been separated by their action on litmus milk—whether turning it pink or only slightly changing or not changing at all the original color.

Examine the colonies on Petri plate at first with the unaided eye, then with a hand magnifying glass or low-power objective, using reflected

FIG. 10.—Series of stab cultures in gelatin, showing modes of growth of different species of bacteria. (*Abbott.*)

and transmitted light alternately. Having determined the presence of two or more different kinds of colonies, make a ring with wax pencil around one or more of each kind of colony, numbering them. The slides or culture tubes used in determining the species of organism present in the plate should bear the same number as that of the colony from which the material was taken. A convenient procedure is to put a loopful of water on a clean cover-glass and to emulsify material from

a colony in it. Then invert over a concave slide without vaselining the circumference of the concavity. After examining for motility, smear out and dry the bacterial preparation. Then fix in the flame and stain with aniline gentian violet for two to five minutes. Wash and mount the preparation in water. Afterward pass through the usual Gram technic.

After this inoculate the various culture media from similar colonies.

In testing for gas production it is better to use the Durham fermentation tube as small amounts of gas may not be easily detected with deep stab cultures into glucose or lactose agar.

If a Durham or Smith tube be not at hand the production of gas may be determined by observing bubble formation on the surface of the sugar bouillon culture. As none of the pathogenic cocci produce gas, fermentation tubes are unnecessary where cocci are to be studied. The litmus milk tube gives data as to acid production.

An important point is to wait at least forty-eight hours (in the case of M. melitensis, four to seven days) before reporting on the cultural findings on the agar or blood-serum slant upon which the material is smeared (pus, exudate, blood, etc.).

Should an organism be encountered in original investigations these requirements as to etiological relationship should be carried out (Koch's postulates). 1. The organism should be constantly present in that particular pathological condition. 2. Such bacteria should be isolated in pure culture from the pathological material. 3. Such pure cultures when inoculated into suitable animals should reproduce the pathological conditions and should be capable of a second isolation in pure culture from such an experimental animal. For various reasons, such as unsuitable animals or artificial media, these requirements are impossible of execution with several organisms which are generally recognized as the causes of certain diseases.

CHAPTER V.

STUDY AND IDENTIFICATION OF BACTERIA—COCCI. KEY AND NOTES.

Streptococcus Forms.—Cells divide to form chains.

I. Gelatin not liquefied.
  A. No capsules on sugar media.
    1. Tendency to form long chains.
      a. Streptococcus pyogenes. (Cocci .7 to 1μ.)
      b. Streptococcus anginosus.
    2. Tendency to form short chains.
      a. Streptococcus salivarius.
      b. Streptococcus fæcalis.
  B. Capsules on sugar media; S. mucosus.
    Very thick capsules on sugar media; S. mesenteroides.

II. Gelatin liquefied.
  Streptococcus coli gracilis. (Cocci quite small—.2 to .4μ. In fæces.)
  A tube-like liquefaction, chains rather long; only slight growth on agar. Constant inhabitant of stools of meat diet.

Sarcina Forms.—Cells divide in three dimensions of space. (Packets.)

A. No pigment production on agar.
  a. Sarcina alba. (Colonies finely granular.)
  b. Sarcina pulmonum.
B. Yellowish pigment.
  a. Sarcina lutea. (Colonies coarsely granular.)
  b. Sarcina flava. (Colonies finely granular.)
C. Rose-red pigment.
  a. Sarcina rosea.

Micrococcus Forms.—Cells divide irregularly in various directions.

I. Gram-positive cocci.
  A. Cocci—round.
    1. Divide in two planes at right angles. Tetrad formation. Merismopedia.
      a. M. tetragenus. Moist white viscid colonies. No liquefaction of gelatin. Capsule.
    2. Divide irregularly. Bunch of grapes arrangement. (Staphylococci.)
      a. Gelatin not liquefied. M. cereus albus.
      b. Gelatin liquefied. { M. (Staphylococcus) pyogenes albus. / M. (Staphylococcus) pyogenes aureus. }
      c. Gelatin very slightly liquefied.
        S. epidermidis albus. (Stitch coccus.)
  B. Cocci—biscuit-shape.
    Diplococcus crassus. (May be mistaken for meningococcus.)
  C. Cocci—lance shape—in pathological material.
    Diplococcus pneumoniæ. Fraenkel's pneumococcus. Capsule.

II. Gram-negative cocci.
  A. Grow only at about incubator temperature.
    1. Grow only on blood or serum media. Gonococcus.
    2. Grow on blood serum media, or glycerine agar.
      a. Diplococcus intracellularis meningitidis. (Produces acid in glucose.)
    3. Grows on ordinary media. Micrococcus melitensis.
  B. Will grow at room temperature as well as at 37° C.
    a. Micrococcus catarrhalis. (Produces alkalinity in glucose media.)

Streptococcus Forms.

Those cocci tending to arrange themselves in chains are usually described as streptoccoci. (Ogston, 1881; Rosenbach, 1884.)

When we consider that certain bacilli at times assume an arrangement which we term strepto-bacilli, yet have no relationship, it would suggest that the matter of chain morphology is simply a characteristic common to many entirely different cocci.

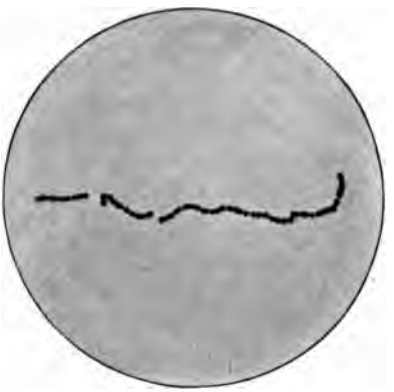

Fig. 11.—Streptococcus pyogenes. (*Kolle and Wassermann.*)

The essential point to bear in mind is that the finding of a streptococcus does not necessarily explain an infection, because normally streptococci are among the organisms most frequently and abundantly found in plates made from normal buccal and nasal secretions. It is well to be very conservative when reporting streptococci as the etiological factor from lesions of the throat or nose.

Probably the most practical point in the differentiation of streptococci, next to that of pathogenicity, is the occurrence of long or short chains, the virulent ones tending to appear in chains of from ten to

twenty cocci, while the normal inhabitants of the nose. mouth and fæces generally tend to be in shorter chains.

As regards virulence, this is exceedingly variable—it is soon lost, but may be restored either by inoculating streptococci along with various other organisms or by passage through successive rabbits. The rabbit is the most susceptible animal and should be inoculated in one of the prominent ear veins. If the needle of the syringe is not inserted in the vein it will be difficult to force in the material and a swelling will immediately show itself.

Besides the morphological and pathogenic variations, Schottmuller has noted differences where these organisms are grown on one part of blood and three to six parts of agar. On this medium Strep. erysipelatis has a hemolytic action, the lacking of the red cells bringing about a more or less clear ring surrounding the colony. The short-chain streptococci do not have a hemolytic halo. The pneumococcus has a greenish zone.

Some of the English authorities have introduced biochemical methods of differentiating: the Strep. pyogenes coagulating milk, reducing neutral red, and producing acid in lactose, saccharose, and mannite media.

S. pyogenes does not produce acid in inulin media while the pneumococcus does.

A freshly prepared solution of sodium taurocholate, 5%, added to an equal amount of a twenty-four-hour bouillon culture of S. pyogenes does not disintegrate the cocci or, at any rate, not within a few minutes. The reverse is true of the pneumococcus.

When we consider the biochemical variations which a single organism, as the colon bacillus, may exhibit, the value of such methods of differentiating may well be questioned. The question of the symbiotic relationship, which, when established between two or more bacteria, may cause harmless organisms to take on virulence, would appear to be a more important consideration.

Almost without exception, human streptococci are Gram-positive. Their colonies are quite small, but distinct and discrete. In appearance the colonies of streptococci and pneumococci are practically identical. In a blood-serum throat culture pneumococcus and streptococcus colonies are the smallest, diphtheria ones are quite small and discrete, but slightly flatter. (Always examine the water of condensation for streptococci.) The sarcina and staphylococcus colonies are much larger.

Streptococci are commonly the cause of diffuse phlegmonous inflam-

mation, while the staphylococci cause circumscribed lesions. Streptococci cause necrosis and do not characteristically produce pus. The importance of the streptococcus as a secondary infection in diphtheria, tuberculosis, small-pox, and even in typhoid fever must always be kept in mind. It is this infection which does not respond to diphtheria antitoxin, and not the diphtheria one.

In nondiphtheritic anginas, puerperal fever, ulcerative endocarditis and coccal enteritis it is the streptococcus which is usually the cause. It has been claimed that acute articular rheumatism is due to a short-chain streptococcus (M. rheumaticus), which is best isolated from material from an acute joint infection, but may also be isolated occasionally from the blood. It produces much acid and clots milk in two days. The growth is described as being more luxuriant than that of S. pyogenes. It is about $.5\mu$ in diameter.

Sarcina Forms.

These are best observed in hanging-drop preparations, when they can be seen as little cubes, like a parcel tied with a string, and by noting them when turning over, it will be seen that they are different from the tetrads which only divide in two directions of space. At times the packet formation is not perfect and it will be difficult to distinguish such as sarcinæ. All sarcinæ stain by Gram. If the staining of sarcinæ be too deep it may obscure the lines of cleavage. Sarcinæ are nonmotile.

Various sarcinæ have been isolated from the stomach, especially when there is stagnation of stomach contents. Sarcinæ have also been found in the intestines. In plates the S. lutea is frequently a contaminating organism, being rather constantly present in the air. The demonstration of sarcina morphology should always be made from liquid media, as bouillon. Urine makes an excellent medium.

Micrococcus Forms.

This grouping includes all cocci which do not show chain or packet formation. It will be found convenient to divide them into two classes according to their staining by Gram. The M. tetragenus, S. pyogenes aureus and the pneumococc sustain by Gram, while the gonococcus, the meningococcus, the M. catarrhalis and the M. melitensis are Gram negative.

M. Tetragenus.—This organism is frequently found associated with other organisms in sputum, especially with tubercle and influenza

bacilli. The colonies are white, slightly smaller than staphylococci and are quite viscid.

It was formerly considered unimportant in disease, but the idea now prevails that it is responsible for many abscesses about the mouth, especially in connection with the teeth. Injected subcutaneously into mice, it produces a septicæmia and death in three or four days. The blood shows great numbers of encapsulated tetrads. It has been reported twice as a cause of septicæmia in man.

Staphylococci.—To cocci dividing irregularly and usually forming masses which are likened to clusters of grapes the term staphylococcus is applied. While there have been experiments which show that by selecting pale portions of a yellow colony, eventually a white colony could be produced, yet, as a practical consideration, it is convenient to consider at least two types of staphylococci: the Staphylococcus pyogenes aureus and the Staphylococcus pyogenes albus. In culturing from the pus of an abscess or furuncle we generally obtain a golden coccus, while in material from the nose or mouth, the staphylococcus colonies are almost invariably white. As regards the common skin coccus, this will be found to produce a white colony. A coccus which very slowly liquefies gelatin and has been supposed to cause stitch abscesses is the S. epidermidis albus.

FIG. 12.—Gelatine culture Staphylococcus aureus one week old. (*Williams.*)

While it is customary to look for a golden colony in the case of organisms showing virulence, yet at times a cream-white colony may develop from cocci of great virulence.

The S. pyogenes citreus is considered as of very feeble pathogenic power. Certain cocci whose colonies have presented a waxy appearance have been designated as S. cereus albus and S. cereus flavus, respectively. They are of vary little practical importance. The staphylococcus pyogenes aureus grows readily at room temperature, but better at 37° C. It coagulates milk and renders bouillon uniformly turbid. It grows on all media, as blood-serum, agar, potato, etc. It has been proposed

to distinguish it from skin staphylococci by its power of producing acid in mannite. Ordinarily the individual cocci are about 1μ in diameter, but they vary greatly in size according to the age of the culture and other conditions. The aureus, as it is frequently called, is not only often found in circumscribed processes, but it is a frequent cause of septicæmia, osteomyelitis, endocarditis, etc.

In infection of bone tissue the staphylococcus is by far the most frequent cause. It is well to remember that insignificant staphylococcal infection may lead to septicæmia. In the tropics, where resistance is often lowered and staphylococcal skin infections common, continued fevers are often septicæmias. It is the organism most frequently concerned in terminal infections. The lowered resistance of the patient permits of their passage through barriers ordinarily resistent. Not only should this be kept in mind when such organisms are isolated at an autopsy, but as well the fact that their entrance may have been agonal or subsequent to death.

The Pneumococcus of Fraenkel.—(Weichselbaum differentiated organisms causing pneumonia in 1886.) This is by far the most common cause of pneumonia, whether it be of the croupous, catarrhal, or septic type. It is also frequently found in meningitis, empyema, endocarditis and otitis media. It should not be confused with the pneumobacillus of Friedländer, which, although possessing a capsule like the pneumococcus, differs from it by being Gram negative, being a bacillus and having large viscid colonies. The pneumococcus is the cause of more than 80% of the cases of pneumonia. It does not grow below 20° C. and is best cultivated on blood-serum, or blood-streaked agar. On plain agar it grows as a very small dew-drop-like colony, which is slightly grayish by reflected light. It produces considerable acid, thus acidifying and usually coagulating litmus milk. It produces acid in inulin media which the streptococcus fails to do. It is smaller and more transparent than a streptococcus colony. In sputum or other pathological material it is best recognized by the presence of a capsule inclosed in which are two lance-shaped cocci with their bases apposed. In artificial culture we rarely get the capsule. It also sometimes grows in short chains like a streptococcus. The best medium for differentiating is the serum of a young rabbit; in this it grows as a diplococcus, while streptococci show chains. The best method of isolating it in pure culture is to inject the sputum into the marginal ear vein of a rabbit or

subcutaneously into a mouse. Death results from septicæmia in about two days and the blood teems with pneumococci. Usually the pneumococcus quickly loses its virulence, and also dies out in a few days unless transferred to fresh media. The best medium for its preservation is rabbit's blood agar; this also maintains the virulence. On this medium the colonies are larger than on agar and they present a greenish appearance. It is a well-known fact that the pneumococcus is a frequent inhabitant of the nasal, pharyngeal, and buccal cavities. The explanation

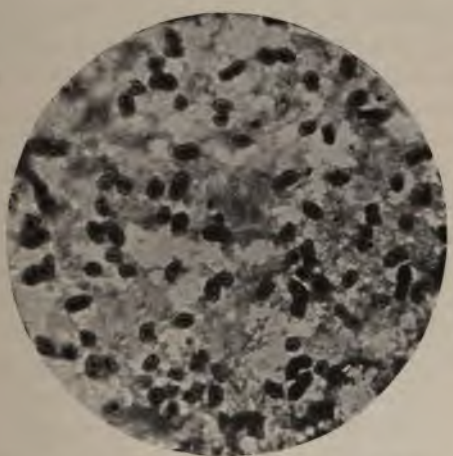

FIG. 13.—Pneumococcus, showing capsule, from pleuritic fluid of infected rabbit, stained by second method of Hiss. (*Williams.*)

of infection is either on the ground of lowered resistance of the patient or enhanced virulence of the organism. Oscar Richardson has reported an organism in cases of lobar pneumonia, cerebrospinal meningitis, mastoid disease, etc., bearing resemblance to both pneumococci and streptococci—the Streptococcus capsulatus. It differs from the pneumococcus in that the colonies on blood-serum are viscid and like irregular flecks of mucus. The characteristic culture is a glucose agar stab. (Reaction must not exceed + .5.) From the line of puncture there are flail-like projections extending outward from one-fifth to one-fourth of an inch. The capsule persists on ordinary culture media. This organism resembles the Streptococcus of Bonome of the French.

Diplococcus Crassus.—This is a Gram positive, kidney-shaped diplococcus, which might be confused with the M. catarrhalis or the meningococcus by ordinary staining methods. In throat cultures I have isolated on several occasions a Gram positive diplococcus which is at times biscuit-shaped, at times irregularly spherical. It possesses two or three metachromatic granules, so that in a Neisser stain for diphtheria the appearance of these granules may be confusing.

Gram Negative Cocci.—It is important to bear in mind that there are many cocci of varying shapes, which in cultures or in smears from the throat, nose or fæces are Gram negative. These are not well classified or described. To distinguish the three important kidney-shaped diplococci, it can be most easily accomplished by cultural methods, using hydrocele agar (ascites or blood agar will answer), ordinary blood-serum and plain agar. The gonococcus will only grow on the hydrocele agar; the meningococcus will grow on this, but likewise grows on ordinary blood-serum. The M. catarrhalis will grow on plain agar as well as on other media.

Other Gram negative organisms of confusing morphology are M. pharyngis siccus, the colonies of which show great crinkly dryness, and M. pharyngis flavus.

Gonococcus (Neisser, 1879).—This organism is characteristically a diplococcus, the separate cocci being plano-convex with their plane surfaces apposed. (Biscuit shape, coffee-bean shape.) They are generally found grouped in masses of several pairs, most strikingly in pus cells or epithelial cells, but also found extracellularly. Except in the height of the disease, there is a great tendency for the organisms to show involution forms, so that instead of biscuit-shaped diplococci we have round, irregular and uneven cocci. It is therefore advisable in searching smears from chronic gonorrhœa to continue the search of Gram-stained specimens until some fairly typical diplococci are found. There is nothing requiring greater discrimination than a diagnosis from such a smear. At the commencement of a gonorrhœa the epithelial cells are abundant and gonococci are found adhering to them or lying free. Later on, at the acme of the discharge (the creamy, abundant discharge), it is in the pus cells we find them and they may be so abundant that 10 to 20% of the pus cells may contain them. In the subacute stage the epithelial cells, which practically disappear when the discharge is so abundant, begin to reappear, and in the chronic stage the epithelial cells are the

chief ones, and are the ones, in which we find an occasional gonococcus, often distorted in shape.

The best method of diagnosis in cases of chronic gonorrhœa is to have the patient drink beer and eat the stimulating food previously interdicted, inject a weak solution of silver nitrate and massage the prostate or seminal vesicles. The smears made from the resulting discharge or centrifuged urine will probably contain gonococci if they are present in the urethra. In the female the favorite sites are the urethra and the cervix uteri. In municipal examinations it is customary

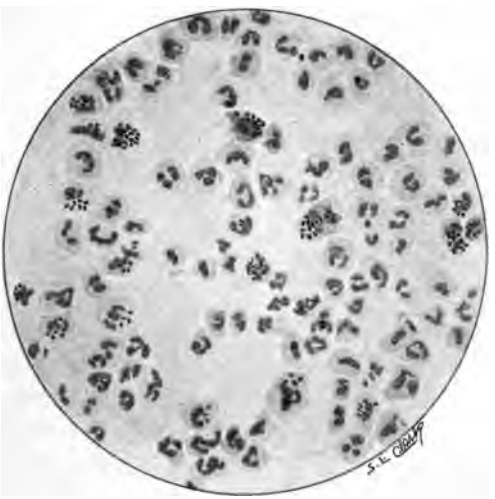

FIG. 14.—Gonococcus. Film from urethral pus. (*Coplin.*)

to make two smears: one from the urethral meatus and a second from the cervix. The vagina is not a suitable soil for their development. In female children it is most often found in the discharge of the vulvovaginitis.

In addition to the genital organs, the gonococcus may at times invade and be isolated from the eye (gonorrhœal ophthalmia), the joints, rarely as a cause of endocarditis and possibly as the factor in septicæmia. Grown upon hydrocele or ascites agar, or blood-streaked agar, or upon blood agar from man or the rabbit, the colonies appear as irregular, minute, dew-drop spots. By the second or third day the involution

forms are abundant, and within four to seven days the culture will probably be found to be dead. Unless frequent transfers are made, it will be best kept alive on blood agar. The organism grows best at 37° C., and will not grow below 25° C. It will not grow on plain or glycerine agar or ordinary blood-serum unless the transfer of considerable pus in inoculating the slants gives it a suitable culture medium. In material from joints, it is in the fibrin flakes that the gonococci are most apt to be found, if found at all.

By heating the blood-streaked agar tubes to 56° C. for twenty minutes (inactivation-destroying complement and hence bactericidal power of blood on slant) greater success in primary cultures will be obtained.

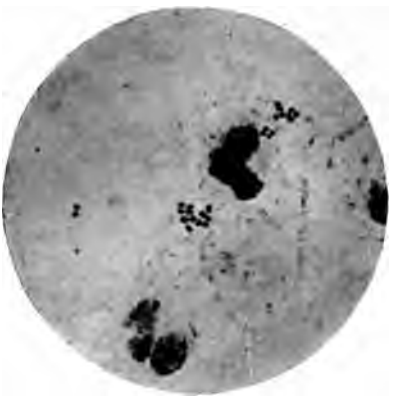

FIG. 15.—Diplococcus intracellularis meningitidis and pus cells. (× 1000.) (*Williams.*)

Diplococcus Intracellularis Meningitidis (Weichselbaum, 1887). —This is the organism of epidemic cerebrospinal meningitis, and is frequently termed the meningococcus. The diplococcus is Gram negative and biscuit-shaped and is, like the gonococcus, chiefly contained in pus cells. It is also found free in the cerebrospinal fluid withdrawn from spotted fever cases. There is a greater tendency to variation in size and shape than is the case with the gonococcus, which latter, in fresh material, show a striking uniformity morphologically. The meningococcus is at times not abundant—early in the case, however, the picture may be similar to that of gonorrhœa.

On blood-serum the colonies appear after twenty-four to forty-eight hours as discrete, very slightly hazy colonies, about one-eighth of an

inch in diameter. On serum agar, as ascites or hydrocele agar, they grow best. Unless considerable cerebrospinal fluid is transferred with the inoculating loop, they do not grow on plain agar. They will grow at times on glycerine agar. It ferments dextrose and only grows at blood temperature, thus distinguishing it from the M. catarrhalis. It is scarcely pathogenic for laboratory animals, with the exception of the mouse and guinea-pig, when intraperitoneal injections but not subcutaneous ones give results. Intradural injections give results. The cultures die out very rapidly, so that it is necessary to make transfers every one or two days. The meningococcus has been isolated from the nasal secretions of patients. The possibility of these organisms being the M. catarrhalis must be considered.

Flexner has shown that in monkeys injections of cultures of D. intracellularis into the spinal canal is followed by migration of the cocci to the nasal cavity both free and in phagocytic leucocytes.

The meningococcus has a very slight resistance to sun or drying so that its aërial transmission seems doubtful. It is supposed to effect an entrance by the nares, thence reaching the cerebral meninges. Infection is probably by direct contagion. Several cases have been reported where with a high leukocytosis the cocci have been found in the polymorphonuclears of blood smears and in cultures from the blood. (In about 25% of cases where from 5 to 10 c.c. are employed.)

By the use of alternate injections into horses of living diplococci, then seven days later of an autolysate made from different strains; seven days later again injecting living diplococci; thus alternating material every week, an antiserum of value has been obtained by Flexner. The immunization requires about one year. In using, withdraw about 20 c.c. of cerebrospinal fluid with a syringe, and then inject, through the same needle, an equal quantity of the serum. The injection is repeated every day for three or four days.

For diagnosis, make smears and cultures from cerebrospinal fluid. The sediment from the centrifuged material gives better results. In tuberculosis the lymphocytes preponderate; in cerebrospinal meningitis the polymorphonuclears.

It has been stated that a point of difference between the phagocytosis with the gonococci and the meningococci is that the meningococci invade and at times destroy the nucleus of the polymorphonuclear, which is not true of gonococci. The appearance of large phagocytic endothelial

cells, often containing polymorphonuclears, in the centrifuged cerebrospinal fluid is a favorable prognostic sign. At times there does not appear to be any relation between the number of phagocytic polymorphonuclears and the severity of the case.

Micrococcus Catarrhalis (Seifert, 1890).—This organism has been specially studied by Lord. It resembles the meningococcus strikingly and can only be differentiated by cultural procedure. It grows on plain agar and at room temperature, and does not produce acid in glucose media. It not only occurs in the nasal secretions of healthy people, but appears to be responsible for certain coryzas and bronchial affections, resembling influenza. It also is responsible for certain epidemics of conjunctivitis.

The original cultures may show only slight growth whereas the subcultures prove luxuriant.

The colonies are larger, more opaque, and have a more irregular wavy border than the round colonies of the meningococcus.

Micrococcus Melitensis (Bruce, 1887).—This is the organism of Malta or Mediterranean fever, sometimes called undulating fever, on account of successive waves of pyrexia running over several months. The disease has a very slight mortality (2%), and the lesions are chiefly of the spleen, which is large and diffluent. The organisms can best be isolated from the spleen.

M. melitensis is only about .3μ in diameter. The characteristics are its very small size and the dew-drop minute colonies on agar, which at incubator temperature only show themselves about the third to the sixth day. It is nonmotile and Gram negative. In bouillon there is a slight turbidity.

The organsim occurs in the peripheral circulation, it having been cultivated from blood very successfully by Eyre. He takes blood at the height of the fever, and in the afternoon. Formerly it was customary to isolate by splenic puncture.

Infection is chiefly by means of the milk of infected goats. The organisms are excreted in the urine of patients, and a diagnostic point is to make plates from the urine. Such urine applied to abraded surfaces causes infection.

The serum of patients shows agglutinating power as early as the fifth day of the disease, and this may persist for years after recovery.

CHAPTER VI.

STUDY AND IDENTIFICATION OF BACTERIA. SPORE-BEARING BACILLI. KEY AND NOTES.

A. **Grow aerobically.**

1. Stab culture in gelatin has branches growing out at right angles to line of stab.
   a. Has no membrane on bouillon or liquefied gelatin. Projecting branches from line of stab only at upper part of line of growth. Absolutely nonmotile. Ends sharply cut across or concave. ANTHRAX GROUP.
   b. Has thick whitish membrane on bouillon and surface of liquefied gelatin. Projecting branches all along the line of stab. Sluggishly motile. MYCOIDES GROUP. (B. mycoides. B. ramosus.)
2. Stab cultures in gelatin do not show projecting branches.
   a. Potato cultures do not become wrinkled. At first slightly moist, later dry and mealy. SUBTILIS GROUP. (Hay bacillus group.) Actively motile.
      The yellow subtilis is at times found in water. The colonies on potato are of a cheese-yellow color. The bacilli are very large and show a sluggish, worm-like motion.
      Cultures of B. megatherium are somewhat similar to B. coli colonies.
   b. Potato cultures at first even growth but after a few days become wrinkled. VULGATUS GROUP. (Potato bacillus.)
      B. vulgatus shows marked wrinkling, like intestinal coils. B. mesentericus show slight wrinkling and a network-like appearance.
      Two water bacilli belonging to this group are the B. mesentericus fuscus (brown growth) and B. mesentericus ruber (red growth).

NOTE.—The following cultural characteristics are common to all the above spore bearers.

1. Liquefaction of gelatin.
2. Milk slowly coagulated with very little change in reaction. Later the coagulum is digested.
3. No gas in either glucose or lactose.
4. No indol.
5. All are Gram positive.
6. All digest blood-serum.

B. **Grow only anaerobically.**

1. Rods very little swollen by centrally situated spores.
   a. Motile. B. œdematis maligni. (Gram negative.)
   b. Nonmotile. B. ærogenes capsulatus. (Capsule.)
2. Spores tend to be situated between center and end.
   a. No liquefaction of gelatin. B. butyricus.
   b. Gelatin liquefied slowly.
      B. botulinus. Milk not coagulated.
      B. anthracis symptomatici.
      B. enteritidis sporogenes. Milk coagulated with abundant gas.
   c. Gelatin liquefied rapidly. B. cadaveris sporogenes. Very motile.
3. Spores situated at end of rod. Drum-stick sporulation. TETANUS GROUP.

The following table taken from Lehmann and Neumann, based on pathogenic effects, is of great practical value. After inoculation of some animal subcutaneously with the suspected material we have:

A. No particular symptoms at site of inoculation.
Absorption of the soluble toxin causing:
(1) General symptoms of tetanus. B. tetani.
(2) Botulism poisoning symptoms. Pupillary symptoms. Paralysis of tongue and pharynx. Cardiac and respiratory failure.

B. Local symptoms marked at site of inoculation. Hemorrhagic emphysematous œdema.
(1) Motile.
(a) Gram negative. B. œdematis maligni.
(b) Gram positive. B. anthracis symptomatici.
(2) Nonmotile.
B. aerogenes capsulatus or B. phlegmonis emphysematosæ.

Spore-bearing Aerobes.

Bacillus Anthracis (Pollender discovered 1849. Davaine recognized nature 1863. Koch proved 1876).—Of the aerobic spore-bearing bacilli this is the only one of particular medical importance.

Fig. 16.—Anthrax bacilli. Cover-glass has been pressed on a colony and then fixed and stained. (*Kolle and Wassermann.*)

Anthrax is an important disease in domestic animals, especially sheep and cattle. The characteristic postmortem change in animals is the greatly enlarged, friable, mushy spleen. Man is much less susceptible than these animals, but is more so than the goat, horse, or pig. The Algerian sheep has a high degree of immunity, as has the white rat.

The brown rat is quite susceptible. The disease in man chiefly occurs among those working with hides, wool, or meat of infected cattle. The two chief types in man are: 1. Malignant pustule and 2. Woolsorter's disease. An intestinal type is also recognized. Malignant pustule results from the inoculation of an abrasion or cut; thus it frequently shows on the arms and the backs of those unloading hides. It first appears as a pimple, the center of which becomes vesicular, then necrotic. A ring of vesicles surrounds this central eschar and a zone of congestion

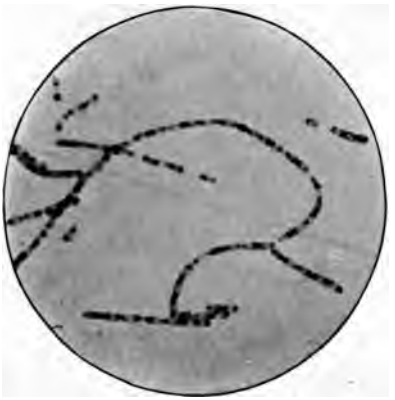

FIG. 17.—Anthrax bacilli growing in a chain and exhibiting spores. (*Kolle and Wassermann.*)

the vesicles. The lymphatics soon become inflamed as well as neighboring glands. If the pustule is not excised and death occurs, there is not much enlargement of the spleen and the bacteria are not abundant in the kidneys, etc., as with animals. Man seems to die from a toxæmia rather than a septicæmia.

In woolsorter's disease there is great swelling and œdema of the bronchial and mediastinal glands. The lungs show œdema, which about the bronchi is hemorrhagic.

The bacillus is 5 to 8μ by 1 to 1 1/2μ. It has square cut or concave ends and is often found in chains. It is Gram positive. Colonies, by interlacing waves of strings of bacteria, show Medusa head appearance. For cultural characteristics see key. Spores develop best at incubator temperature.

Stiles thinks that animals are infected by eating the bones of animals which have died of anthrax, cutting buccal mucous membrane, and so becoming infected. Spores do not form in an intact animal body, but they do form after a postmortem or the disintegration of the body by maggots. For this reason it is better not to open up the body of the animal, but to make the diagnosis by cutting off an ear. Dried spores will live for years and will withstand boiling temperature for hours.

In vaccinating animals against anthrax, Pasteur used two vaccines.

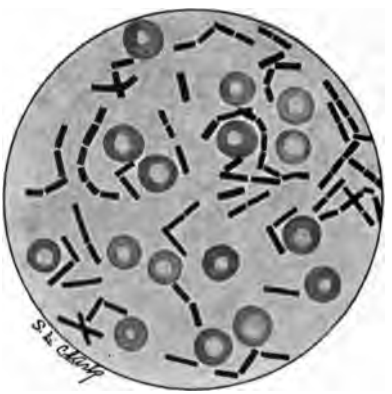

FIG. 18.—Bacillus anthracis in blood of rabbit. (*Coplin.*)

The first is attenuated fifteen days at 42.5° C. The second, attenuated for only ten days, is given twelve days later.

In taking material from a malignant pustule before excision, be careful not to manipulate it roughly, as bacteria may enter the circulation. Make cover-glass preparations, staining by Gram. Make culture on agar. Blood cultures are usually only positive later in the disease. Inoculate a guinea-pig or a mouse subcutaneously.

SPORE-BEARING ANAEROBES.

There are three very important pathogens in this group—that of malignant œdema; that of botulism, and the organism of tetanus.

The B. enteritidis sporogenes is of importance in connection with indications of fæcal contamination of water. In connection with B. aerogenes capsulatus, there is some question as to whether the extensive

œdema produced by it may not usually be from a terminal or cadaveric infection.

To Cultivate Anaerobes.—Probably the apparatus giving the most perfect anaerobic conditions is the Novy jar, in which the air has been replaced by hydrogen. The difficulties attending the method are:

1. Unless a special apparatus (Kipp's) is at hand, there may be difficulty in preventing the sulphuric acid from frothing over when poured on the zinc. It should, at first, be added in small quantities at a time—well diluted (1 to 6).
2. Various wash-bottles are required: one containing silver nitrate solution for traces of AsH_3 and one with lead acetate for H_2S and another with pyrogallic acid and caustic soda for any oxygen that may come over.
3. Mixtures of hydrogen and air explode. Consequently, in determining whether all air has been expelled and in its place an atmosphere of hydrogen exists, it is necessary to see if the escaping gas burns with a blue flame. Unless this is collected in a test-tube and examined, we may have an explosion.
4. Except in a large laboratory, where the aparatus is set up and ready for use, too much time would be requircd.
5. Simpler methods appear to give as good results.

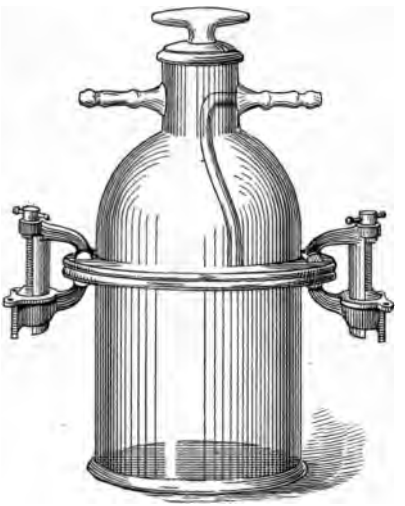

FIG. 19.—Novy jar.

The Method of Liborius.—In this it is necessary to have a test-tube containing about 4 inches of a 1% glucose agar. Glucose acts as a reducing agent and furnishes energy. It is convenient to add about 1/10 of 1% of sulphindigotate of soda; the loss of the blue color at the site of the colony enabling us to pick them out. The tube of agar should be boiled just before using to expel remaining oxygen from the tube.

Now rapidly bring down the temperature to about 42° C., by placing the tube in cold water, and inoculate the material to be examined. A second or third tube may be inoculated from the first, just as in ordinary diluting methods for plate cultures. Having inoculated the tubes, solidify them as quickly as possible, using tap water or ice-water. The anaerobic growth develops in the depths of the medium. Some pour a little sterile vaseline or paraffin or additional agar on the top of the medium in the tube as a seal from the air. Others have recommended the inoculation of some aerobe, as B. prodigiosus, on the surface. This latter method is not advisable. A deep stab culture is often sufficient.

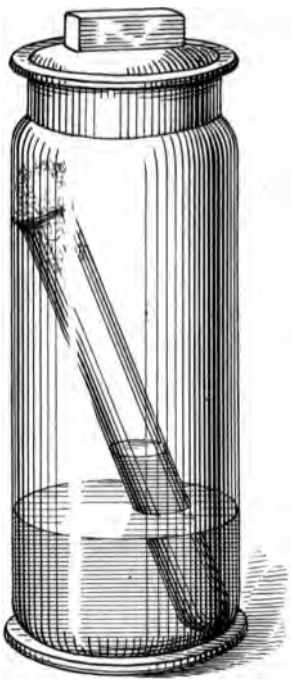

FIG. 20.—Arrangement of tubes for cultivation of anaerobes by Buchner's method. (*Williams.*)

The Method of Buchner.—In this method one gram each of pyrogallic acid and caustic potash or soda for every 100 c.c. of space in the vessel containing the culture is used to absorb the oxygen. It is convenient to drop in the pyrogallic acid; then put in place the inoculated tubes or plates; then quickly pouring in the amount of caustic soda, in a 10% aqueous solution, to immediately close the containing vessel. A large test-tube in which a smaller one containing the inoculated medium is placed, and which may be closed by a rubber stopper, is very convenient. A good rubber-band fruit jar is satisfactory. A desiccator may be used for plates. An excellent method for anaerobic plates, either in a desiccator with the pyrogallic acid and caustic soda, or less satisfactorily in the open air, is to sterilize the parts of the Petri dish inverted; that is, the smaller part is put bottom downward in the inverted cover (as one would set one tumbler in another). Then, in using, unwrap the Petri dish, lift up the inner part, pour in the inoculated medium into the upturned cover. Then immediately press down the inner dish, spreading out a thin film of medium between the two bottoms.

J. H. Wright's Method.—Make a deep stab culture in glucose agar or gelatin, preferably boiling the media before inoculating. Then flame the cotton plug and press it down into the tube so that the top lies about three-fourths of an inch below the mouth of the test-tube. Next fill in about one-fourth of an inch with pyrogallic acid; then add 2 or 3 c.c. of a 10% solution of caustic soda, and quickly insert a rubber stopper. This method is one of the most convenient and practical, and is to be strongly recommended.

Method of Vignal.—In this a section of glass tubing (1/4 in.) is drawn out at either end, as in making a bacteriological pipette, with a mouth-piece containing a cotton plug. The liquid agar or gelatin is then inoculated and the medium drawn up into the tube. In a very small flame the capillary narrowings are sealed off, and we have inside the tube very satisfactory anaerobic conditions. To get at the colonies, file a place on the tube and break at this point.*

B. Œdematis Maligni (Pasteur, 1877).—This is the vibrion septique of Pasteur. It is found in garden soil and in street sweepings. It is the cause of an acute cellular necrosis attended with serous sanguinolent exudation and with more or less emphysema. The organism only becomes generalized in the blood about the time of death and postmortem. Therefore, it is not a septicæmia, as is anthrax. The bacillus is an organism about the size of anthrax (7μ by .8), but is narrower and does not have the same square cut or dimpled ends. Furthermore, it is motile, Gram negative and an anaerobe. The guinea-pig is very susceptible, and about the time of death and post-mortem there may be seen long flexile motile filaments, 15 to 40 μ long, which move among the blood cells as a serpent in the grass (Pasteur).

In cultures it grows out very slightly from the line of stab, giving a jagged granular line, differing from tetanus. Spores form best at 37° C. —requiring about forty-eight hours. It liquefies gelatin. In examining an exudate from a suspected case, note the presence of spores centrally situated. Inoculate a guinea-pig. Death occurs in about two days. There is intense hemorrhagic emphysematous œdema at the site of inoculation. The subcutaneous tissue contains fluid and gas. There

* To obtain material for examination and isolation in pure culture from the deep agar stab-tube, it is best to loosen the medium at the sides of the tube with a heated platinum spud or a flattened copper wire. Then shake the mass out into a sterile Petri dish. It is dangerous to break the tubes with a hammer as some do.

is present the foul odor of an anaerobe. Examine for the long filaments showing flowing motility. Be sure to stain by Gram. (Negative.) For cultures, heat the material (either from a wound or from a guinea-pig) which shows spores to a temperature of 80° C. for from fifteen minutes to one hour. Then inoculate glucose agar stab culture and grow anaerobically. Courmont differentiates anthrax from malignant œdema by injecting into ear-vein of rabbit. The injection of malignant œdema in this way, instead of subcutaneously, tends to immunize.

B. Botulinus (Van Ermengem, 1896).—This is the organism which produces botulism, a form of meat poisoning. It is a spore-bearing

FIG. 21.—Bacillus of botulism. (*Kolle and Wassermann.*)

anaerobe and must not be confused with another organism associated with meat poisoning—the B. enteritidis of Gärtner.

In botulism the meat becomes infected after the animal has been slaughtered; in Gärtner meat poisoning the cow meat was infected at the time of slaughter—it was from a sick animal. Thorough cooking of the meat protects against botulism but not certainly against Gärtner meat poisoning.

There are dysphagia, paralysis of eye-muscles, and cardiac and respiratory symptoms (medulla). The symptoms are due to the elaboration of a soluble toxin of the same nature as that of diphtheria and tetanus. There is no fever and consciousness is preserved. It has been isolated from sausage and ham. It is a large bacillus—5 to $10\mu \times 1\mu$. It is motile and stains by Gram. It produces gas in glucose media. It

grows best at 22° and only slightly at 37°—hence it is dangerous only from its soluble toxin, the bacilli not developing to any extent in the body.

When the toxin is introduced, it requires a period of incubation of twelve to twenty hours. An important point is that ham may not appear decomposed and yet contain many bacilli and much toxin. It is a very potent toxin—as little as one-thousandth of a c.c. may kill a guinea-pig. In man the toxin is apparently absorbed from the alimentary canal. For diagnosis inject an infusion of the ham or sausage

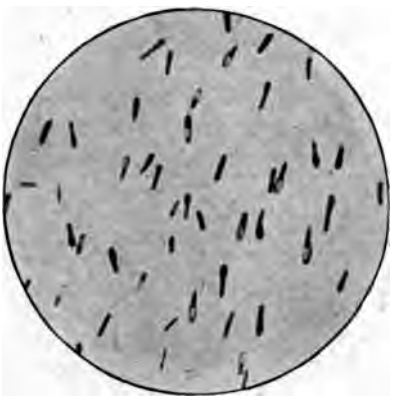

FIG. 22.—Symptomatic anthrax (Rauschbrand) bacilli showing spores. (*Kolle and Wassermann.*)

which was eaten of into a guinea-pig, and characteristic pupillary symptoms with death by cardiac and respiratory failure will result.

Cultures may be made in glucose agar. The characteristic point is the production of a powerful soluble toxin which produces symptoms when no bacilli are present.

B. Tetani (Nicolaier, 1885; Kitasato, 1889).—This is the most important organism of the anaerobic spore bearers. Its characteristics are the tetanic symptoms produced by the toxin and the strictly terminal drum-stick spores. Spores are difficult to find in material from wounds infected with tetanus, but readily develop in cultures. Prior to the formation of spores the organism is a long thin bacillus ($4 \times .4\mu$). It is motile and Gram positive. It liquefies gelatin slowly and does not coagulate milk. Theobald Smith recommends growing it in fermenta-

tion tubes containing ordinary bouillon, but to which a piece of the liver or spleen of a rabbit or guinea-pig has been introduced at the junction of the closed arm and the open bulb. By this method spores develop rapidly in from twenty-four to thirty-six hours. Sporulation is most rapid at 37° C. As there is always liability to postmortem invasion of viscera by ordinary saprophytes, Smith recommends that great care be taken not to handle the animal roughly in chloroforming and in pinching off pieces of the organ at autopsy. The animal must be healthy, and the tubes to which the piece of tissue is added must be proven sterile by incubation. Smith calls attention to the uncertainty of the temperature

FIG. 23.—Tetanus bacilli showing end spores. (*Kolle and Wassermann.*)

at which tetanus spores are killed. He shows that some require temperature only possible with an autoclave. In view of the danger of tetanus, it is advisable to carefully autoclave all material going into bacterial vaccines, such as salt solution, bottles for holding, etc.

Tetanus seems to grow better in symbiosis with aerobes; hence a lacerated dirty wound with its probable contamination with various cocci, etc., and its difficulty of sterilization, offers a favorable soil. The tetanus bacillus gives rise to one of the most powerful poisons known; it is a soluble toxin like diphtheria toxin, and it is estimated that 1/300 of a grain is fatal for man.

Rosenau has established an antitoxin unit for tetanus which has the power of neutralizing one thousand minimal lethal doses. Practically,

it is ten times the least quantity of antitetanic serum necessary to protect the life of a 350 grams guinea-pig from a test dose of tetanus toxin furnished by the hygienic laboratory. The necessity of some definite unit is apparent when tests have shown that serum stated to contain six million units per c.c. only had a value of 90 of the official American units. Consequently it is a unit ten times as neutralizing as the diphtheria antitoxin one. The antitoxin of tetanus is less efficient than that of diphtheria for the following reasons:

1. There is about three times as great affinity *in vitro* between diphtheria toxin and antitoxin as is the case with tetanus.

2. The tetanus toxin has greater affinity for nerve cells than for antitoxin.

3. Treatment with antitoxin is successful after symptoms of diphtheria appear. With tetanus it is almost hopeless after the disease shows itself. Hence the importance of the early bacteriological examination of material from a suspicious wound (rusty nail).

4. The tetanus toxin ascends by way of the axis cylinder, and the antitoxin being in the circulating fluids cannot reach it, whereas with diphtheria both toxin and antitoxin are in the circulation. Diphtheria also selects the cells of parenchymatous and lymphatic organs which are more tolerant of injury than the nerve cells. The dose of tetanus antitoxin as a prophylactic is 1500 units; as a curative agent 5000 to 20,000 units. Recent experience shows that it should be injected intravenously when symptoms have manifested themselves.

That the disease is due to toxin is shown not only experimentally, but also if spores are carefully freed of all toxin by washing, and then introduced they do not cause tetanus—the polymorphonuclears engulfing them. The importance of the presence of ordinary pus cocci in a tetanus wound may be that the activity in phagocytizing them allows the tetanus bacillus to escape phagocytosis. This would also explain the importance of necrotic tissue in a lacerated wound—the phagocytes taking this up instead of tetanus bacilli. The toxin is digested by the alimentary canal juices and infection by that atrium is improbable. The infection occurs especially through skin wounds, and also from those of mucous membrane. While tetanus is like diphtheria, a disease in which the bacilli are localized and do not spread, yet recently Richardson has obtained tetanus bacilli in pure culture from the tributary lymphatic glands of a "rusty nail" wound of foot. The cultures inoculated into

root of tail of a white rat caused the rat's death in forty-eight hours with typical "seal gait" attitude of tetanus in rats.

FIG. 24.—B. aerogenes capsulatus agar culture showing gas formation. (*Williams.*)

The usual period before symptoms occur is fifteen days. The shorter the period of incubation, the more probably fatal the disease. The horse is the most susceptible animal, next the guinea-pig, then the mouse. Fowls are practically immune.

In examining for tetanus, scrape out the material from the suspected wound with a sterile Volkmann spoon and put it in a tube containing blood-serum. Place this in an incubator. We have here the principle of the septic tank—the cocci and other aerobes grow luxuriantly and enable the tetanus bacillus to develop. From day to day smell the culture, and if an odor similar to the penetrating, sour, foul smell of the stools of a man who has been on a debauch be detected, it is suspicious. The nondevelopment of a foul odor is against tetanus. Also make smears from the material and examine for drum-stick spores. If these are found, heat the material to 80° C. for one-half hour, to kill non-sporing aerobes and facultative anaerobes, and then inoculate a deep glucose agar tube and cultivate by Wright's method. The fusiform lateral outgrowth about the middle of the stab is characteristic.

B. Aerogenes Capsulatus (Welch, 1891).—This bacillus is apparently widely distributed. It is possibly the same organism as Klein's B. enteritidis sporogenes, which is constantly

present in fæces. It is a large capsulated organism, which does not form chains. Spores are produced on blood-serum. These are frequently absent on other media. It is questioned whether its pathogenicity is other than exceedingly feeble, the presence of the bacillus in emphysematous findings at postmortem being attributed to terminal or cadaveric invasion. Cases, however, in the Philippines, have been reported following carabao horn wounds, in which most serious and fatal results attended emphysematous lesions showing this bacillus.

In milk cultures we have coagulation and from the subsequent development of gas the disruption of the coagulum into shreds. An odor of butyric acid is developed.

It is the cause of "foamy organs" occasionally present at autopsy.

The best method of diagnosis is to inoculate the culture or material into the ear vein of a rabbit, kill it and then incubate the body at 37° C. Gas is generated in the organs in a few hours.

Achalme isolated a large bacillus from a fatal case of rheumatism which is now considered as having no relation to acute rheumatism and which was probably B. aerogenes capsulatus.

CHAPTER VII.

STUDY AND IDENTIFICATION OF BACTERIA. MYCOBACTERIA AND CORYNEBACTERIA. KEY AND NOTES.

Key for Bacilli.—Having branching characteristics, as shown by parallelism, branching, curving forms, V-shapes, clubbing at ends, segmental staining, etc.

Acid-fast. Mycobacterium. { Cultures more or less wrinkled and dry. More like moulds.

I. Grow rapidly on ordinary media at room temperature.
   Examples: Timothy grass bacillus of Moeller (B. phlei).
   Mist bacillus. Butter bacilli as reported by (1) Rabinowitsch and (2) Petri.

II. Only grow at about incubator temperature. Scanty growth or none at all on ordinary media. Media of preference are: (a) solidified blood-serum, (b) glycerine agar, (c) glycerine potato and (d) egg media.
   1. Cultures fairly moist, luxuriant, and flat. Op. temp. 43° C.
      a. Bacillus of avian tuberculosis.
   2. Cultures scanty, wrinkled, and dry. Appear in ten to fourteen days. Op. temp. 38° C. Bacilli longer, narrower, more regular in outline and staining than bovine; vacuolation more marked (2.5μ). Smear from organs of inoculated guinea-pig shows few bacilli. Less virulent for rabbits.
      a. Bacillus of human tuberculosis.
      Cultures as above, but even more scanty. Bacilli shorter, thicker, less vacuolated (1.5μ). Smear from organs of guinea-pig shows many bacilli.
      b. Bovine tubercle bacilli.
   3. Very difficult to cultivate (Czaplewski).
      Smegma bacilli of various animals.

III. Noncultivable by ordinary methods. Cultivable in symbiosis with amœbæ. (Clegg.)
   1. B. lepræ. Found chiefly in nasal mucus and in juice from lepra tubercles. Less often in nerve leprosy.

Nonacid-fast. Corynebacterium. { Colonies more flat and moist. Like other bacteria.

I. Do not stain by Gram's method.
   1. B. mallei (Glanders). Characteristic culture is that on potato. Growth like layer of honey by third day. Becomes darker in color, until on eighth day is reddish brown or opaque with greenish-yellow margin.

II. Gram positive.
   1. Very luxuriant growth on ordinary media. Colonies often yellow to brownish. B. pseudodiphtheriæ. Shorter, thicker and stain uniformly.
   2. Moderate growth on ordinary media. B. diphtheriæ. Best media are blood-serum (Löffler's) or glycerine agar. Has metachromatic granules at poles.
   3. Scanty and slow growth on nutrient media. B. xerosis.

The Group of Acid-fast Branching Bacilli.

There is a large and ever-increasing number of organisms which have the same staining reactions as the tubercle bacilli, but which differ in four important essentials of:

1. Growing readily on any media.
2. Showing more or less abundant growth or colonies in twenty-four hours.
3. Having no pathogenic power for guinea-pigs when inoculated subcutaneously.
4. Not requiring body temperature for development, but growing at room temperature.

Many of these organisms, if injected intraperitoneally into guinea-pigs will produce a peritonitis with false membrane. Some also produce granulation tissue nodules which may be confused with true tubercles. For this reason it is well to study the lesion in experimental tuberculosis in the guinea-pig. Injected subcutaneously, on either or both sides of the posterior abdomen with the needle pointing toward the inguinal glands, we may have caseation and ulceration at the site of inoculation. The glands in relation enlarge and caseate. Smears from these show T. B. The marked and characteristic change is the enormous enlargement of the spleen, which is studded with grayish and yellow tubercles. Make smears and cultures from the spleen. The death of the guinea-pig usually occurs in about two months. The lesions may be looked for at three to five weeks.

These nonpathogenic acid-fast bacilli are of greatest importance by reason of their possible confusion with the true tubercle bacilli. Their colonies correspond more or less with different types of tubercle bacilli colonies, being either dry and wrinkled like human, or moist and irregularly flat as avian. Eventually the moist colonies become dry and wrinkled. They have been isolated from:

1. Butter and milk.
2. From grasses, especially in timothy grass infusion.
3. In various excretions of animals, as in dung, urine, etc.
4. Normally in man—from skin, nasal mucus, cerumen, and tonsillar exudate.

It is important to remember that such organisms have very rarely been reported from pulmonary lesions, and when present they have been considered as probably causative.

FIG. 25.—Bacillus tuberculosis; glycerine agar-agar culture, several months old. (*Curtis.*)

The present view is that the finding of tubercle bacilli in sputum has practically as great value as it had before we knew of these various acid-fast bacteria.

Tubercle Bacillus (Koch, 1882).—This is a rather long, narrow rod, 3 x .3μ. In the human type it tends to show a beaded appearance, this not being due to spores, however. In the bovine type the staining is more solid, the organism shorter and thicker, and shows even a more scanty growth than human T. B. It has been established that many of the tuberculous affections of man, especially those of the skin, bone, and mesenteric glands, are of the bovine type, while, as a rule, pulmonary and laryngeal lesions are of the human type. Experiments by various commissions in different countries have shown that human and bovine types are very closely related and that not only may a bovine strain affect man, but that human T. B. may infect young calves. As bacilli of the bovine type have frequently been reported in intestinal and mesenteric tuberculosis of children it shows the importance of sterilizing cows' milk. Koch considers human infection from bovine sources as of very rare occurrence.

Recent statistics (Beitzke) show tuberculous lesions in 58% of adults at autopsy—Naegli's figures were about 90%.

It is a question whether the avian type is absolutely distinct; many experiments having indicated the impossibility of infecting fowls with human T. B. Nocard, by inserting col-

lodion sacs containing bouillon suspensions of human T. B., claims to have changed these to the avian type. The avian type grows at 43° C. fairly luxuriantly, as a moist, more or less spreading culture. Morphologically they are like the human type, but show less tendency to form compact masses. Very pleomorphic. Have been reported from sputum of man (doubtful).

There is also a fish tuberculosis. This organism grows much more rapidly than the other types (three to four days), and grows best at 24° C., growth ceasing at 36° C. The colonies are round and moist.

The best culture media for primary cultures are blood-serum or, better, a mixture of yolk of egg and glycerine agar. Dorset's egg medium is also used. In subcultures, either glycerine agar, glycerine potato, or glycerine bouillon make good media. In inoculating media from tuberculous material, as, say, from a tuberculous gland or, more practically, from the spleen of a guinea-pig, the material must be thoroughly disintegrated or rubbed on the surface of the media so that individual bacilli may rest on the surface of the culture media. In growing in flasks in glycerine bouillon a surface growth is desired. The cylindrical flask of Koch gives a better support to the pellicle than an Erlenmeyer one. In inoculating, a scale of such a surface growth or a grain from the growth on a slant should be deposited on the surface of the glycerine bouillon in the flask.

In asmuch as the filtrate from cultures has little toxic effect, the poison is assumed to be intracellular.

Koch's old tuberculin, which was simply a concentrated glycerine bouillon culture, is now principally used in veterinary diagnosis. It was prepared as follows:

After four to six weeks the surface growth begins to sink to the bottom of the flask. This fully developed culture is evaporated over a water bath at 80° C. to one-tenth the original volume. It is then filtered, the final product containing about 40% of glycerine.

Koch's tuberculin "R" or new tuberculin was introduced in 1897. In this, virulent bacilli are dried *in vacuo*, ground up in water and centrifuged. The first supernatant fluid is discarded. Subsequent trituration and centrifugalization, preserving each time the supernatant suspension, gives the new tuberculin. It has been found at times to contain virulent T. B.

Koch's bazillen emulsion has been recently introduced by Koch.

This is simply a suspension of ground-up bacilli in 20% glycerine solution. Another preparation is the bouillon filtrate of Denys. This is the unheated filtrate of broth cultures of human T. B.

In the use of T. R. and of bazillen emulsion, Sir A. Wright recommends doses of 1/4000 of a milligram, and he rarely goes beyond 1/1000 of a milligram in treatment. These products come in 1 c.c. bottles containing 10 mg. of bacillary material. It is convenient to remove 1/10 of a c.c., containing 1 mg. Add this to 10 c.c. of glycerine salt solution with 1/4% of lysol. Each c.c. contains 1/10 mg. One c.c. of this stock solution added to 99 c.c. of salt solution, with 1/4% of lysol, would give a working solution, each c.c. of which would contain 1/1000 mg. of tuberculin.

For diagnostic reactions we have, besides the method of injecting tuberculin and noting presence or absence of fever, four more recent diagnostic tuberculin tests: 1. Variations in opsonic index. 2. Instillation into one eye of a drop of 1/2% or 1% solution of purified tuberculin. Reaction is shown by redness, especially of inner canthus, in twelve to twenty-four hours (Calmette). A previous instillation may sensitize a nontuberculous case and a second application of the drop may give an erroneous diagnosis. 3. The cutaneous inoculation method (similar to ordinary vaccination methods). Scarify two small areas on the arm (1/10 inch in diameter), about 2 inches apart. Rub in one drop of old tuberculin. As a control scarify a spot midway and to one side of the others and rub in one drop of 0.5% carbolic glycerine. The appearance of bright red papules in twenty-four hours indicates reaction (von Pirquet). This is the method of preference. 4. Ointment tuberculin test. Rub in 50% ointment of tuberculin in lanolin. Reaction is shown by dermatitis with reddened papules in twenty-four to forty-eight hours (Moro). 5. Inoculation of bovine and human tuberculin to diagnose type of infection (Detre). Of questionable value.

Ebright injects the suspected material into the subcutaneous tissue of one side of the abdomen of three guinea-pigs. At the end of one week an injection into the other side of the abdomen of one of the guinea-pigs of 1/4 c.c. tuberculin is given. Twenty-four hours later smears are made from the original site of inoculation and examined for tubercle bacilli. If negative this is repeated with a second guinea-pig at the end of the second week and finally at the end of the third week with the third guinea-pig.

In staining it is better to use the Ziehl-Neelsen method, decolorizing with 3% hydrochloric acid in 95% alcohol. The alcohol, for all practical purposes, enables us to eliminate the smegma and similar bacilli, these being decolorized by such treatment. There are two objections to the Gabbett method, where decolorizer and counterstain are combined: 1. We cannot judge of the degree of decolorization—we are working in the dark; and 2. the matter of elimination of smegma bacilli is impossible.

Pappenheim's method, in which corallin and methylene blue are dissolved in alcohol, does not appear to have any advantage over acid alcohol. As a practical point when the question of tuberculosis of the genito-urinary tract is involved, inoculate a guinea-pig with urinary sediment.

It must be remembered that in young cultures of tubercle bacilli many of the rods are nonacid-fast, taking the blue of the counterstain, while older rods are acid-fast. This frequently causes suspicion of a contaminated culture.

Bacillus Lepræ (Hansen, 1874).—This is the cause of leprosy. In nodular leprosy the organism is readily and in the greatest abundance found in the juice of the tubercles of the skin, nasal and pharyngeal mucosa.

The earliest lesion is probably a nasal ulcer at the junction of the bony and cartilaginous septum. Scrapings from this ulcer may give an early diagnosis.

In the skin they are chiefly found in the derma, especially in connective-tissue cells and endothelial cells. They are also found in the glands in relation to the superficial lesions. The bacilli are found in smaller numbers in the liver and spleen. In anæsthetic or nerve leprosy they are found in small numbers in the granuloma tissue which affects the interstitial connective tissue of the peripheral nerves. Also, rarely, in the anæsthetic spots of nerve leprosy.

In morphology and staining reactions they are almost identical with the tubercle bacillus. The main points of distinction are: 1. The fact of the leprosy bacilli being found in enormous numbers, especially in large vacuolated cells (lepra cells), and lying in the lymph spaces. They are frequently beaded and lie in masses which have been likened to a bundle of cigars tied together. It may be necessary to examine for long periods of time, smears made from tuberculous lesions of skin before

finding a single organism. 2. Leprosy bacilli have not surely been cultivated. 3. Injected into guinea-pigs, they do not produce lesions.

Recent work has shown that the Wassermann test gives positive findings in practically all cases of tubercular leprosy but is generally negative in anæsthetic cases.

Recently a leprosy-like disease of rats has been reported in which there are two types: 1. A skin affection and 2. a glandular one. In this disease—acid-fast bacilli, alike in all respects to leprosy bacilli, have been found. Deane has obtained a diphtheroid-like organism in culture, which is nonacid-fast. This same finding has been obtained in cultures considered positive in human leprosy. Quite recently it has been claimed that the leprosy bacillus has been cultivated by excising aseptically the subcutaneous portion of lepromata and dropping the leprous tissue into salt solution, the resulting growth being like a streptothrix. This was the basis of the Nastin treatment which is now more or less discredited. In 1909 Clegg reported that by smearing plates containing amœbæ with spleen pulp of lepers (in which the bacilli were abundant) he obtained growth of an acid-fast bacillus. He was able to carry on these organisms in subculture for several generations. A vaccine made from these bacilli does not seem to have been successful. For diagnosis we should use both smears from the nasal mucus and from ulcerated lepromata or from the scrapings from intact tubercles. Some advise centrifuging with salt solution, but this is rarely necessary. The best method is to excise a small portion of skin or mucous membrane, fix it in absolute alcohol or Zenker's fluid. Cut thin sections in paraffin. Stain with carbol-fuchsin, decolorize with acid alcohol, and then stain with hæmatoxylin. This gives the location of the bacilli. This is also a good method for tuberculous tissues. It is claimed that the B. lepræ stains more easily and loses its color more rapidly than the tubercle bacillus. Some prefer to stain the leprosy bacillus by Gram's method, it as well as the tubercle bacillus being Gram positive.

Nonacid-fast Branching Bacilli.

Bacillus Mallei (Löffler and Shutz, 1882).—This is the cause of a rather common disease of horses. When affecting the superficial lymphatic glands, it is termed "farcy;" when producing ulceration of nasal mucous membrane, the term "glanders" is used.

In man there are two types of glanders—chronic and acute. In the chronic form an abrasion becomes infected from contact with glanders material and an intractable foul discharging ulceration results. This may persist for months with lymphatic involvement or may become acute. The acute form may also develop from the start and the cases are usually diagnosed as pyæmia. Death invariably results in acute glanders. The bacillus is a narrow, slightly curved rod, about 3 x .3μ. It is nonmotile and Gram negative. It at times presents a beaded appearance. In subculture on agar or blood-serum the growth is somewhat like typhoid but more translucent. In original cultures from pus or tissues the colonies may not show themselves for forty-eight hours. The characteristic culture is that on potato. Grown at 37° C., we have a light brown mucilaginous growth, which by the end of a week spreads out and takes a café au lait color. The potato assumes a dirty-brown color. This and the inoculation of a guinea-pig are the chief diagnostic measures. If the material is injected intraperitoneally into a male guinea-pig, marked swelling of the testicles is noted within forty-eight hours.

The best stains are carbol thionin and formol fuchsin. In sections stained with carbol thionin the bacilli are apt to be decolorized by the subsequent passage of the section through alcohol and xylol. This may be avoided by blotting carefully after the thionin, then clearing with xylol or some oil and mounting. Nicolle's tannin method is a good one.

Mallein is prepared by sterilizing cultures that have grown in glycerine bouillon for about a month by means of heat (100° C.). The dead culture is then filtered through a Berkefeld filter and the filtrate constitutes mallein. It is chiefly used as a means of diagnosing the disease in horses. The reaction consists in rise of temperature and local œdema. The dose is about 1 c.c.

Bacillus Diphtheriæ (Klebs discovered, 1883; Löffler cultivated, 1884).—The diphtheria bacillus is found not only in the false membrane which is so characteristic of the disease, but may be found in abundance in the more or less abundant secretions of nose and pharynx. In studying the epidemiology of diphtheria, especial attention must be given to the examination of nasal discharges.

Infection of the larynx and middle ear are not very rare. The mucous membrane of the vagina or the conjunctiva may also be infected.

The B. diphtheriæ may be in pure culture lying entangled in the fibrin meshes or contained within leukocytes in the membrane or be associated with staphylococci, pneumococci, or especially streptococci. These latter complicate unfavorably and cause the suppurative conditions about the neck. In fatal cases the diphtheria bacillus may be found in the lungs. Ordinarily, however, it remains entirely local and does not get into the circulation or viscera.

It produces soluble absorbable poisons which are designated toxin in the case of the one responsible for the acute intoxication, paren-

FIG. 26.—Bacillus of diphtheria. (× 1000.) (*Williams.*)

chymatous degeneration and death; and toxone for the poison which produces œdema at the site of inoculation and postdiphtheritic palsy. The injection of the soluble poisons alone without the bacilli produce the symptoms of the disease.

The bacilli tend to appear as slightly curved rods, showing varying irregularities in staining, as banding or beading, and in particular the presence at either end of small, deeply staining dots (metachromatic granules). These are well seen with Löffler's blue, but better with Neisser's method. In culture they also show swelling at one or both ends or clubbing. In secretions or in culture they show V-shapes or false branching and, what is most characteristic, the parallelism—four

or five bacilli lying side by side like palisades. Being a Gram positive organism while the majority of the other pathogenic bacteria are Gram negative, it is of greatest importance to stain smears by this method. It is not so strongly tenacious of the gentian violet as the cocci, so decolorization should not be carried too far.

The best medium for growing it is Löffler's blood-serum. Coagulated white of egg answers equally well, as will a hard-boiled egg—the shell at one end being cracked and the white cut with a sterile knife. This smooth side is then inoculated and the egg placed cut side downward in a sherry glass. If an incubator is not at hand a tube may be

FIG. 27.—Diphtheria bacilli involution forms. (*Kolle and Wassermann.*)

carried next the body in a pocket. The bacillus grows better on glycerine agar than on plain agar. On such plates they appear as small, coarsely granular colonies with a central dark area. In size the colonies resemble the streptococcus. On blood-serum the colonies are larger—1/12 to 1/8 inch in diameter.

The diphtheria bacillus grows luxuriantly on blood agar and like the streptococcus pyogenes has a yellowish laked zone around the colony. The Hofman and the Xerosis bacillus do not seem to have this hæmolytic power. In bouillon it tends to form a surface growth. It is at the surface that the toxin function is most marked, hence in growing diphtheria for toxin formation we use Fernbach flasks which expose a large surface to the air. It is a marked acid producer—bouillon with a +1 reaction becoming +2.5 to +3 in thirty-six hours. The filtrate from a two- or

three-weeks-old broth culture is highly toxic, and is usually referred to as diphtheria toxin. It is used in injecting horses to produce antitoxin. Ehrlich uses as a standard to measure the toxicity of toxin the minimal lethal dose (M. L. D.). This is the amount of toxin which will kill a 350-gram guinea-pig in just four days. Some toxins have been produced whose M. L. D. was 1/500 c.c., so that 1 c.c. of such toxin would kill 500 guinea-pigs. Theoretically, the measure of an antitoxin unit is the capacity of neutralizing 200 units of a pure toxin. (On exposure to light, etc., toxin loses its toxic power and is termed toxoid.) Inasmuch, however, as toxone and toxoid are also present, we may practically consider an antitoxin, or immunizing unit (*i.e.*, Immunitätseinheit) as about capable of making innocuous 100 M. L. D.

In the preparation of antitoxin horses are employed; the method being to inject the bouillon filtrate or toxin subcutaneously at weekly intervals for a period of three or four months. When each c.c. of the serum of the horse is found to contain about 250 to 500 antitoxin units the horse is bled from the jugular vein. Some sera contain as much as 1300 units.

Methods of purifying and concentrating antitoxin are now employed by certain makers.

As a curative measure, from 2500 to 5000 units should be injected. If the injection is delayed or the case very serious the dose should be 10,000 units. As much as 50,000 units has been given in severe cases. The prophylactic dose is 500 units.

Sudden death after administration of antitoxin has been reported in cases of status lymphaticus. (See anaphylaxis).

In obtaining material from a throat, be sure that an antiseptic gargle has not been used just prior to taking the throat swab. The part of the swab which touched the membrane or suspicious spot should come in contact with the serum slant. This is best accomplished by revolving the swab. An immediate diagnosis is possible in probably 35% of cases by making a smear from a piece of membrane. In doing this Neisser's stain is the most satisfactory.

If there is any doubt about the nature of an organism in a throat culture, always stain: 1. with Löffler's alkaline methylene blue for two minutes; 2. with Gram's method, being careful not to carry the decolorization too far, and 3. by Neisser's method. With Löffler's you obtain a picture which, after a little experience, is characteristic; at times the polar bodies

show as intense blue spots in the lighter blue bacillus. One is liable to confuse cocci lying side by side for diphtheria bacilli with segmental or banded staining. This difficulty is not apparent when Gram's staining is used. This gives us great information, as the diphtheria and the pseudodiphtheria are the only small Gram positive bacilli usually found in the mouth. The cocci are also well brought out. Neisser's stain gives a picture which, when satisfactory, is almost absolutely characteristic. You have the bright blue dots lying at either end of the light brownish-yellow rods. When first isolated from a throat, the diphtheria bacillus is apt to stain characteristically by Neisser. Later on, in subculture,

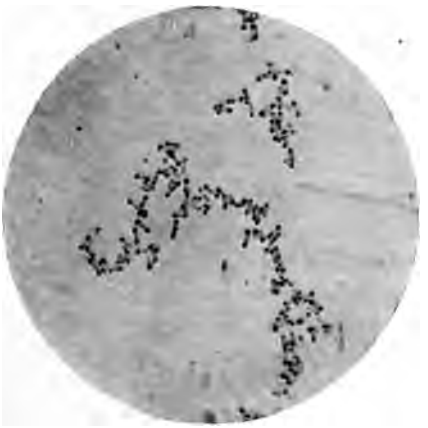

FIG. 28.—B. diphtheria stained by Neisser's method. (*Williams.*)

there may be no staining of the polar bodies. Neisser originally recommended five seconds' application, with an intermediate washing, for each of his two solutions. Thirty seconds for each is probably preferable. Some authorities recommend five to thirty minutes. It is well to bear in mind that about 2% of the people in apparent health carry diphtheria bacilli in their throats.

It is essential when a question exists as to the nature of a diphtheria-like organism to test it as to virulence. While there are exceptions, especially in freshly isolated colonies, yet as a rule a severe infection yields virulent organisms and *vice versa*. Pure cultures are best obtained by streaking material from the throat on glycerine agar plates. From an isolated colony inoculate a tube of bouillon. From such a twenty-

four-hour-old culture inoculate a guinea-pig with two or three drops subcutaneously in the shaven abdomen. Escherich considers a fatal result with 1.5 c.c. of such a bouillon culture a satisfactory test as to virulence. After death, which occurs in two or three days, the adrenals are enlarged and hæmorrhagic.

Diphtheroid Bacilli. Pseudodiphtheria Bacillus. Hofman's Bacillus.—Under these terms various Gram positive bacilli have been described as occurring in nose and in skin diseases:

1. They very rarely give the blue dot staining at the two ends. Exceptionally they may give a dot at one end. Neisser attaches importance to the dots at both ends as showing diphtheria.
2. They tend to stain solidly or at most with only a single unstained segment. They are shorter, thicker, and do not curve so gracefully as the true diphtheria bacillus. They are stockier.
3. They produce very little acid in sugar media, not one-half that produced by true diphtheria.
4. They are nonpathogenic for guinea-pigs.
5. Many of them grow quite luxuriantly and often show chromogenic power.

Xerosis Bacillus.—This organism is frequently found in normal conjunctival discharges. There is question as to its pathogenesis, and the finding of this organism should not exclude the previous presence of strictly pathogenic organisms, such as the gonococcus or the Koch-Weeks. It resembles the diphtheria bacillus in being Gram positive and showing parallelism, but differs 1. in being nonvirulent for guinea-pigs; 2. in requiring about two days for the appearance of colonies; 3. in not showing Neisser's granule staining, and 4. in producing very little acid in sugar media.

CHAPTER VIII.

STUDY AND IDENTIFICATION OF BACTERIA. GRAM NEGATIVE BACILLI. KEY AND NOTES.

KEY to the recognition of nonspore-bearing, nonchromogenic, non-Gram-staining, nonbranching bacilli.

(NOTE.—Some books say that the proteus group is Gram positive. It is, however, usually negative.)

Do not grow on ordinary media. Require blood agar (hæmophilic bacteria), serum agar, or blood-serum.

Minute dew-drop colonies.

1. Influenza bacillus. Requires blood media.
2. Koch-Weeks bacillus (conjunctivitis). Serum agar best medium.
3. Müller's bacillus of trachoma. Like Koch-Weeks bacillus, but easier to cultivate.
4. Morax diplobacillus of conjunctivitis. Grows well and produces little pits of liquefaction in Löffler's blood-serum.
5. Pseudoinfluenza bacillus (whooping-cough?).
6. Ducrey's bacillus (soft chancre). Requires almost pure blood. Forms chains.

Grow well on ordinary media.

I. Cultures in litmus milk. PINK.

A. Nonmotile.

Lactis aerogenes group. B. lactis aerogenes.

Produce gas in glucose, lactose, and saccharose. No liquefaction of gelatin. Short, stubby bacteria.

B. Motile.

1. Nonliquefaction of gelatin.

a. B. coli group. Coagulation of milk. No subsequent peptonization. Gas in glucose and lactose, none in saccharose. Indol produced. Neutral red reduced.

2. Liquefaction of gelatin.

a. B. cloacæ group. Gas in glucose, slight in lactose. Slow coagulation of milk. Subsequent peptonization.

II. Cultures in litmus milk. LILAC.

A. Nonmotile bacilli.

1. No gas generated in glucose or lactose bouillon.

a. Hemorrhagic septicæmia group. These are oval bacilli with tendency to bipolar staining.

Colonies smaller and less opaque than those of B. coli.

Examples: B. pestis, B. suisepticus, B. choleræ gallinarum (chicken cholera).

B. pseudotuberculosis rodentium (very similar to plague).

B. pestis is absolutely nonmotile, does not liquefy gelatin, does not produce indol, produces slight acid in glucose but not in lactose bouillon.

b. Dysentery group. Colonies similar to those of B. coli.
Divided into two classes according as mannite is acted on:
Those not giving acid—nonacid group—(Shiga-Kruse).
Those giving acid—acid group—(Flexner-Strong).

2. Gas generated in glucose bouillon not in lactose.
a. Friedländer group. Give very viscid, porcelain-like colonies.
Tendency to capsule formation in favorable media.
Examples: B. pneumoniæ, B. capsulatus mucosus, B. rhinoscleromatis.

B. Motile bacilli.
1. Do not liquefy gelatin.
a. Do not produce gas in either glucose or lactose bouillon.
Typhoid, or Eberth group. No indol. No coagulation of milk. No reduction of neutral red.
b. Gas generated in glucose, not in lactose media. Milk not coagulated. Neutral red reduced.
Gärtner group. This includes:
Pathogenic types for man; as, B. enteritidis, B. icteroides, B. paratyphoid B, B. psittacosis. Nonpathogenic for man; as B. choleræ suum (hog cholera).
2. Liquefy gelatin.
a. Proteus group. Colonies at first round later amœboid, spreading. Produce gas in glucose, not in lactose. Produces foul odor.
B. zopfii type of Proteus group does not liquefy gelatin; colonies at first round, later amœboid, spreading. Foul odor in cultures. Gelatin stab shows lateral branching.

Gram-Negative Bacilli Requiring Special Media.

Bacillus Influenzæ (Pfeiffer, 1892).—This organism is the type of the so-called hæmophilic bacteria—organisms whose growth is restricted to media containing hæmoglobin. The influenza bacillus seems to grow better on slants freshly streaked with blood than on those which have been made for some time, and they appear to grow better on this surface smear of blood than on a mixture of agar and blood.

The influenza bacilli are most likely to be isolated from the sputum of bronchopneumonia due to this organism. It has also frequently been found in the nasal secretions of influenza patients. Exceptionally, it is present in the blood, and has been isolated in cases of meningitis. It is a very small bacillus which in sputum tends to show itself in aggregations, especially centering about M. tetragenus. It stains rather faintly when compared with cocci, so that a smear of sputum stained with formol fuchsin shows a deep violet staining for the M. tetragenus or other cocci, and scattered around in a clump-like aggregation we see these minute, rather faintly stained rods. They also tend to stain more deeply at either end, so that they sometimes appear as diplococci. Gram's method, counterstaining with formol fuchsin, is excellent for their

demonstration. The red bacilli and the violet-black cocci are easily distinguished.

To cultivate them, rub the sputum, or at autopsy the material from a lung, on a slant smeared with human blood (pigeon's blood is also satisfactory), and then without sterilizing the loop, inoculate a second blood slant; then a third, and possibly a fourth. The colonies appear as very minute dewdrop-like points which seem to run into each other in a wave-like way. To test such colonies we should transfer a single colony to plain agar and blood-serum, trying not to carry over any blood. If the least trace of blood is carried over, they may grow on agar or blood-serum. Organisms resembling the influenza bacillus have been isolated

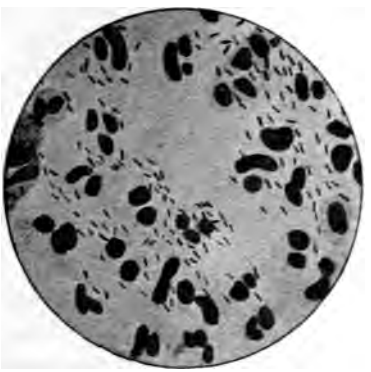

FIG. 29.—The Koch-Weeks Bacillus. (*Hansell and Sweet.*)

from whooping-cough. Such organisms have also been found in the fauces of well persons. In many epidemics of influenza the bacillus has not been isolated, or success has obtained in only a small proportion of the cases. Etiological factors in conditions more or less resembling influenza may be the Streptococcus, Pneumococcus, or M. catarrhalis. The influenza bacillus seems to grow best in symbiosis with some other organism, especially with S. pyogenes aureus.

Koch-Weeks Bacillus (Koch, 1883).—This produces a severe conjunctivitis. It is very common in Egypt and is also a frequent cause of conjunctivitis in the Philippines and in temperate climates.

Smears made from conjunctival secretion show large numbers of small Gram negative bacilli, especially contained within pus cells, but also

lying free. They are more difficult to cultivate than the influenza bacillus, but the same general methods hold. The vitality of this organism is very slight so that almost immediate transference of material is necessary. Flies are an important factor in Egypt. The period of incubation is very short, twelve to thirty-six hours. The best medium is a mixture of glycerine agar and hydrocele or ascites fluid. At first we rarely obtain pure cultures. The colonies are dewdrop-like and first show themselves in about thirty-six hours in incubator cultures.

Diplobacillus of Morax.—This organism causes mild conjunctivitis chiefly at the internal angle of the eye. They are about 1 or 2μ long and tend to occur in pairs or short chains. Some claim that they are Gram positive. After two or three days on blood-serum rather marked involution forms occur. While usually causing a more or less chronic conjunctivitis they may at times produce a keratitis.

Bacillus of Chancroid (Ducrey, 1889).—These are short coccobacilli, occurring chiefly in chains. They show bipolar staining. They grow best in a mixture of blood and bouillon.

Gram Negative Bacilli Growing on Ordinary Media.

Bacillus Pneumoniæ (Friedländer, 1882).—This organism is responsible for about 5% of the cases of pneumonia. It is usually termed the pneumobacillus to distinguish it from the pneumococcus; at other times Friedländer's bacillus. The name of Fraenkel attaches to the pneumococcus. Morphologically, it is a short, thick bacillus, and in pathological material, as sputum, shows a wide capsule. It is nonmotile and Gram negative. The colonies on agar are of a pearly whiteness and are markedly viscid. On potato it shows a thick viscid growth containing gas bubbles. The characteristic culture is the nail culture of a gelatin stab. The growth at the surface is heaped up like a round-headed nail, the line of puncture resembling the shaft of the nail. It does not liquefy gelatin. It does not produce indol, and does not produce gas in lactose bouillon—differences from the colon bacillus—with which it may be confused in cultures, as it does not then possess a capsule. If in doubt, inject a mouse at the root of the tail. Death from septicæmia occurs in two days. The peritoneum is sticky and numerous capsulated bacilli are present in the blood and organs. The organisims which have been isolated from rhinoscleroma and ozœna

are practically identical with the B. pneumoniæ. This group of organisms are generally referred to as the Friedländer group. Similar organisms have been isolated from the discharges of middle-ear diseases and in anginas. Cases have been reported where the B. pneumoniæ was the cause of septicæmia in man.

Bacillus Pestis (Kitasato, Yersin, 1894).—This is the organism of plague. It is the member of the group of hæmorrhagic septicæmias (Pasteurelloses), from which man suffers. It is primarily a disease of rats. Other Pasteurelloses are chicken cholera and swine plague. Where the plague bacilli are found chiefly in the glands, we have bubonic

FIG. 30.—Colonies of plague bacilli forty-eight hours old. (*Kolle and Wassermann.*)

plague; when in lungs, pneumonic plague; when localized in the skin and subcutaneous tissue, the cellulo-cutaneous; and when as a septicæmia, septicæmic plague. An intestinal type is recognized by some authors. It must be remembered that in all forms of plague the lymphatic glands show hemorrhagic œdema; it is in bubonic plague, however, that the areas of necrosis with periglandular œdema are prominent. Where the symptoms are slight, mainly buboes, the term pestis minor is sometimes used; the typical disease being termed pestis major. In pneumonic plague we have a bronchopneumonia.

In smears from material from buboes, from sputum, or in blood smears, as well as from blood or spleen smears from experimental animals, we obtain the typical morphology of a coccobacillus ($1.5 \times .5\mu$) with

very characteristic bipolar staining; there being an intermediate, unstained area. Very characteristic also is the appearance in these smears of degenerate types which stain feebly and show coccoid and inflated oval types. The presence of these involution forms associated with typical bacilli is almost diagnostic for one with experience. Inoculating tubes of plain agar and 3% salt agar with this same material, we obtain in plain agar cultures organisms which are typically small, fairly slender rods, which do not stain characteristically at each end and are not oval. The smear obtained from the salt agar presents most remarkable involution forms—coccoid; root-shaped, sausage-shaped forms, ranging from

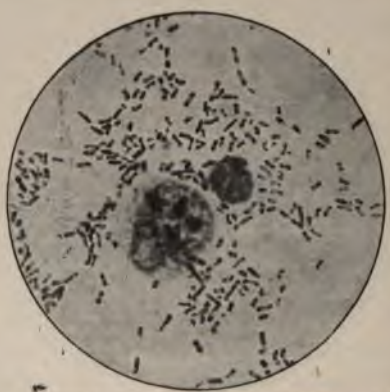

FIG. 31.—Pest bacilli from spleen of rat. (*Kolle and Wassermann.*)

three to twelve microns in length, more resembling cultures of moulds than bacteria. Another point is that on the inoculated plain agar we are in doubt at the end of twenty-four hours whether the dewdrop-like colonies are really bacterial colonies or only condensation particles. By the second day, however, these colonies have an opaque grayish appearance, so that now, instead of questioning the presence of a culture, we consider the possibility of contamination.

Blood cultures in septicæmic plague may show from 5 to 500,000 per c.c. Smears from the blood in such cases are positive in only about 17%.

The plague bacillus grows well at room temperature—its optimum temperature being 30° instead of 37° C., as is usual with pathogens. Next to the salt agar culture, the most characteristic one is the stalactite growth in bouillon containing oil drops on its surface. The culture

grows downward from the under surface of the oil drops as a powdery thread. These are very fragile, and as the slightest jar breaks them, it is difficult to obtain this cultural characteristic.

While Klein states that B. coli, proteus vulgaris and, in particular, B. bristolensis may be mistaken for plague bacilli, if bipolar staining alone be relied upon, yet it is B. pseudotuberculosis rodentium which may confuse an experienced worker. While this latter is only moderately pathogenic for rats yet the fact that rats may be immunized to B. pestis by inoculation with B. pseudotuberculosis rodentium brings up the suspicion of identity of the two organisms. In diagnosing always

FIG. 32.—Pest bacillus involution forms produced by growing on 3% salt agar. (*Kolle and Wassermann.*)

use animal experimentation. Owing to the difficulty in emulsifying plague bacilli, agglutination tests are not satisfactory. Albrecht and Ghon have shown that by smearing material into the intact, shaven skin of a guinea-pig, infection occurs. This is the most crucial test. Mice inoculated at the root of the tail quickly succumb. Rats, this being primarily a disease of rats, are of course susceptible. Other rodents, as squirrels, are susceptible. It has been suggested that a rodent, the Siberian marmot, might be the starting-point of plague outbreaks. In natural plague of rats, the lesions which establish a diagnosis even without the aid of a microscope are subcutaneous injection of the flaps of the abdominal walls as they are turned back, fluid in the pleural cavities, œdematous hæmorrhagic swelling of the neck glands, and in

particular a creamy, mottled appearance of the liver. The neck glands are chiefly involved because the flea prefers to inhabit the skin of the neck. Smears from the spleen will show the oval bacilli.

A chronic rat plague, which may be a factor in keeping up the disease, is characterized by enlargement of the spleen and the presence within it of nodules containing plague bacilli. McCoy has noted that the frequency of the cervical bubo in rats, noted by the Indian Commission (72%), was not found in California. The glands show periglandular injection and infiltration as well as enlargement.

Recent investigations in India have definitely determined the fact that the flea (Loemopsylla cheopis) is the intermediary in the transmission of plague from rat to rat and from rat to man. In primary pneumonic plague the infective nature is very great and appears to be by the respiratory atrium. This was the terrifying type of plague in the black death of the fourteenth century.

For diagnosis make smears and cultures from material drawn from a bubo by a syringe. (At a later stage, when softening begins, there may not be any bacilli present.) Also, if pneumonic plague, from the sputum. Blood cultures and even blood smears may be employed in septicæmic plague. Formol fuchsin and Archibald's stain make satisfactory stains. Always inoculate a guinea-pig with the material either by rubbing it in with a glass spatula on the shaven skin or by subcutaneous injection. For prophylaxis the most important method is that of Haffkine. Stalactite bouillon cultures of plague are grown for five to six weeks. These are killed by a temperature of 65° C. for one hour. Lysol (1/4%) is added to the preparation and from 0.5 to 4 c.c. injected, according to the age and size of the individual treated. Susceptibility is reduced about one-fourth, and of those attacked after previous vaccination, the mortality is only about one-fourth of what it is among the noninoculated. Strong prepares a prophylactic vaccine from living plague cultures rendered avirulent. Yersin's serum, made by injecting horses with dead plague cultures and afterward with living ones, is of value prophylactically and has possibly considerable curative power.

The Eberth, Gartner and Escherich Groups.—From a standpoint of cultures in litmus milk and sugar bouillon we can divide the organisms related to typhoid at one extreme and the colon at the other into three groups.

1. *The* Eberth or typhoid group. There are three important path-

ogens in this group: the B. typhosus, the B. dysenteriæ, and the B. fæcalis alkaligenes. The color of litmus milk is practically unaltered, and there is no gas production in either glucose or lactose bouillon. No coagulation of milk. No reduction of neutral red. The B. typhosus and the B. fæcalis alkaligenes are actively motile, while the B. dysenteriæ is nonmotile or practically so.

During the first twenty-four to forty-eight hours there is a moderate acid production by typhoid, so that the milk culture is less blue, while with the B. fæcalis alkaligenes the alkalinity is intensified from the start, so that the blue color is deepened.

2. The Gärtner or hog cholera group. Besides organisms important for animals and probably at times for man, such as B. choleræ suum and B. psittacosis and B. icteroides (interesting historically as having been reported as the cause of yellow fever by Sanarelli), we have two pathogens: 1. B. enteritidis (Gärtner's bacillus) and 2. B. paratyphoid B. In this connection it may be stated that the present view is that hog cholera is caused by an ultra-microscopic organism and not by the B. choleræ suum.

These organisms cannot be separated culturally, but only by immunity reactions. They do not turn litmus milk pink. They produce gas in glucose bouillon, but not in lactose. They very powerfully reduce neutral red with the production of a yellowish fluorescence. They do not coagulate milk. There is a transient acidity in the litmus milk, but becoming shortly afterward alkaline, the lilac-blue color is intensified. Both organisms are motile.

3. The Escherich or colon group. These turn litmus milk pink, coagulate milk, reduce neutral red, and show varying degrees of motility. The three groups of organisms just described are non-liquefiers of gelatin. Two intestinal organisms, the B. cloacæ and the Proteus vulgaris, differ in liquefying gelatin.

Bacillus Typhosus (Eberth, 1880; Gaffky, 1884).—This organism may be isolated from the stools, urine, and the blood of typhoid patients. At postmortem it can be best isolated from the spleen, but is also present in Peyer's patches which have not ulcerated. When ulceration has occurred contamination with B. coli is almost sure. Cultures may be obtained from the liver also. In sections made from spleen the Gram negative bacilli are apt to be decolorized. Thionin, then blotting and clearing in oil or xylol, shows the clumps of bacilli lying between the cells.

Formerly it was supposed that by the differences in the thickness of the film of a colony or by its varying shades of grayish-blue, we possess data of importance in differentiating typhoid from related organisms.

The colonies look like grapevine leaves.

Growth on potato was also considered as affording information. At present, the biochemical reactions give us information assisting in differentiation, and the agglutination and bacteriolytic phenomena, the final diagnosis. The various plating media are considered under the study of fæces.

FIG. 33.—Seventy-two-hour-old culture of typhoid bacillus on gelatin. (*Kolle and Wassermann.*)

Not only do we find hyperplasia of the endothelial cells in the lymphoid tissue of Peyer's patches and the mesenteric glands and the spleen, with subsequent necroses, but focal necroses of the same character are found in the liver.

A striking feature of the pathology of typhoid fever is the long-continued persistence of the organisms in the gall-bladder and elsewhere. It is beginning to be believed that a previous typhoid infection, possibly so mild as to have passed unnoticed, is at the basis of gall-bladder infections and resulting gall-stones. Various bone infections, especially osteomyelitis, have shown the typhoid bacilli in pure culture. Formerly it was supposed that the typhoid bacillus brought about its lesions by a local infection centered in the ileum. The present view is that typhoid bacilli effect an entrance into the blood stream through some lymphoid channel, as by tonsil or other alimentary lymphoid structure.

They develop in the general lymphatic system, the spleen in particular where they are protected from the bactericidal power of the blood. After a time, however, approximately the period of incubation, they become so abundant in these lymphatic organs that they are carried over into the general circulation. Then as a result of bacteriolysis the intracellular toxins are liberated and symptoms develop. If bacteriolysis takes place other than in the blood we have various suppurative processes. As a result of the formation of antibodies, the development in spleen, etc., is checked but should these immunity reactions become less potent relapses, may occur or various local infections manifest themselves.

As the bacilli do not multiply to any extent in the blood itself the disease cannot be considered as a typical septicæmia but as a bacteriæmia.

Typhoid bacilli can be isolated from the blood during the latter period of incubation and rarely after the tenth day. It is a practical point that the time to isolate the bacteria from the blood is in the first days of the attack. The diagnosis by agglutination is only expected after the seventh to tenth day. Agglutination may not appear until during convalescence, and in about 5% of the cases it is absent. It, as a rule, disappears within a year. Very little success has been obtained with curative sera. Chantamesse, by treating horses with a filtrate from cultures of typhoid bacilli on splenic pulp and human defibrinated blood, claimed to have obtained a curative serum possessing antitoxic power. Wright's method of prophylactic inoculation is now being employed in the British army with apparent success. In this, twenty-four- to forty-eight-hour-old cultures are killed at 53°C.; 1/4% of lysol is then added. An injection of 500 million bacteria is made at the first inoculation, and ten days later an injection of one billion. The British prefer to inject subcutaneously in the infraclavicular region and at the insertion of the deltoid. The Germans consider three injections as conferring greater immunity.

Typhoid vaccines sterilized with 0.5% of phenol appear to keep much longer and to have a higher immunizing power than those prepared by sterilization with heat and subsequent addition of the antiseptic.

A very important discovery is that certain persons, who may have had only a slight febrile attack, may eliminate typhoid bacilli for years in their fæces (typhoid carriers). The bacilli are also eliminated for

considerable periods in the urine. Distinction is now being made between acute carriers (convalescents) and chronic carriers

Typhoid carriers are said to maintain a high opsonic index. Ordinarily, it is very difficult to isolate typhoid bacilli from the stools. For laboratory diagnosis, blood cultures during the first week and agglutination tests during the second week and onward are the practical methods.

Instead of the usual blood cultures one may use the clot in the Wright U-tube for culturing and the serum remaining after centrifugalization

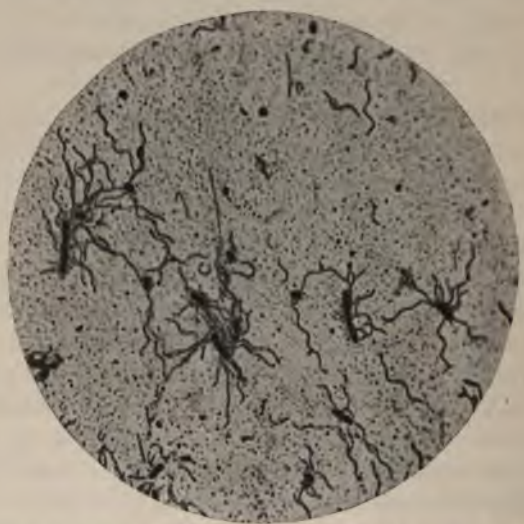

FIG. 34.—Bacillus of typhoid fever, stained by Löffler's method to show flagella. (× 1000.) (*Williams.*)

for the Widal test (clot culture). B. typhosus appears in the blood in relapses. Kayser considered that about 27% of cases of typhoid in Strasburg were caused by raw milk, 17% by contaminated water, 17% by contact with typhoid, and 10% were due to typhoid carriers. Other cases were due to infected food, and about 13% were of origin impossible to determine. These latter may have been due to unrecognized typhoid carriers. He does not attach the same importance to fly dissemination as do American authors.

***The Gartner* or Meat-poisoning Group.**—Under this designation

may be considered the organisms which cause gastrointestinal disorders of varying degrees, infection with which is usually brought about by the ingestion of meat obtained from diseased cattle. Unless the meat is thoroughly cooked the bacilli in the interior may not be killed.

In this group may be placed B. enteritidis, the typical meat-poisoning organism, B. paratyphoid B, B. Danysz, B. Aertryck, B. typhi murium and B. suipestifer.

These organisms are alike morphologically and culturally and show quite a tendency to bipolar staining and reduction of neutral red with fluorescence in forty-eight hours. B. paratyphoid B, B. Aertryck and B. suipestifer are alike from an agglutination stand-point, while B. entertidis and B. Danysz show similarity in this respect. B. paratyphoid A stands by itself.

Paratyphoid Bacilli (Achard and Bensaude, 1896; Schottmüller, 1901).—Cases resembling mild attacks of typhoid occasionally show agglutination for paratyphoid bacilli. These organisms have also been isolated from the blood, as with typhoid. Two types have been recognized: the paratyphoid A and the paratyphoid B. The latter occurs in 80% of such cases. Culturally, paratyphoid B. cannot be separated from Gärtner's bacillus. In paratyphoid A there is less gas produced in glucose bouillon than with paratyphoid B, and the primary acidity of litmus milk is not succeeded by a subsequent alkalinity. It does not seem practical to draw a fine distinction between these two strains.

Bacillus Enteritidis (Gärtner, 1888). This organism has been frequently isolated from cases of gastroenteritis from ingestion of infected meat. This organism is very pathogenic for laboratory animals, producing a hæmorrhagic enteritis and at times a septicæmia. Where meat has been contaminated with Gärtner's bacillus toxins may have been produced, and symptoms of poisoning with acute gastroenteritis would occur shortly after ingestion. It is interesting to note that this toxin is not destroyed by the boiling temperature, thus differing from the toxin of the other important meat-poisoning (botulism) bacillus—B. botulinus—which is rendered innocuous by a temperature of 65° or 70° C. If there is only a little toxin introduced with the contaminated meat, the symptoms will be delayed one or two days. Such organisms have been isolated in pure culture from cases with high fever, marked intestinal derangement, with considerable blood in the rather fluid stools.

In two cases studied the disease was at first diagnosed as a severe typhoid infection. Klein thinks the organism of Danysz's virus (to kill rats during plague epidemics) may be identical with B. enteritidis.

Proteus Vulgaris.—This organism is often encountered in plates made from fæces. It is common in decaying meat or cheese, and cases of even fatal poisoning with marked gastrointestinal symptoms and cardiac failure have been reported. At times it is the cause of cystitis. The colonies on agar are moist and unevenly spreading (amœboid). The bacilli are very motile and, as a rule, Gram negative. It digests blood-serum and is a rapid liquefier of gelatin. The cultures generally have a putrefactive odor. In infective jaundice (Weil's disease) this organism has been reported as the cause. Organisms of this group were formerly designated as B. termo.

Bacillus Dysenteriæ (Shiga, 1898).—Dysentery bacilli produce a coagulation necrosis of the mucous membrane of the large intestine and occasionally of the lower part of the ileum. Polymorphonuclears are contained in the fibrin exudate. It was formerly thought that these lesions were of local origin, but the present view is that toxins are produced which, being absorbed, are eliminated by the large intestine with resulting necrosis. Flexner, by injecting rabbits intravenously with a toxic autolysate, produced characteristic intestinal lesions. There are two main types of dysentery bacilli:

1. Those producing acid in mannite media—the acid strains (Flexner-Strong types).

2. Those not developing acid in mannite (Shiga-Kruse types). Ohno finds that fermentative reactions do not correspond to immunity ones. Thus an acid strain used to immunize a horse may produce a serum more specific for a nonacid strain. The Shiga type is very toxic in cultures, while the Flexner type does not seem to possess a soluble toxin. At the Lister Institute injections of a soluble toxin produced a serum of marked antitoxic power. Such a dysentery serum, which is probably both antitoxic and antimicrobic, is of curative value. Shiga immunized horses with polyvalent cultures and obtained a polyvalent serum which has reduced the death-rate about one-third.

The dysentery bacillus is present in the mucous stools during the first five or six days to the disease. By the tenth day it has probably disappeared. In all cultural respects the dysentery bacillus resembles the typhoid, and the only practical method of distinguishing these two

organisms, other than by agglutination reactions, is by the nonmotility or exceedingly slight motility of the dysentery bacillus. The dysentery bacilli do not form those threads or whip-like filaments so characteristic of typhoid cultures. The dysentery bacillus is not found in the blood and hence is not eliminated in the urine. It is found in mesenteric glands. In dysentery patients agglutination phenomena do not show themselves until about the twelfth day from the onset. Hence, this procedure is of no particular value in diagnosis. It is of value, however, to identify an organism isolated from the stools at the commencement of the attack, using serum from an immunized animal or a human convalescent for the agglutination test.

There seems to be very little agglutination power in the serum of convalescents.

Morgan has reported as the cause of certain cases of bacillary dysentery a bacillus known as B. Morgan, No. 1. It is motile, produces indol, and in glucose bouillon gives a very slight amount of gas.

Park and Collins, in 1904, separated various strains of dysentery bacilli into three groups:

1. Original Shiga strain. No indol. No fermentation of mannite, maltose, or saccharose.
2. A type fermenting mannite, but not maltose or saccharose. No indol.
3. An indol producer which actively ferments mannite and maltose.

B. COLI, B. LACTIS AEROGENES, B. CLOACÆ.

While the colon bacillus chiefly inhabits the large intestine, the B. lactis aerogenes is to be found in the upper part of the small intestine. While they may be separated on the ground of motility, yet it is by the greater fermentative activity of the B. lactis aerogenes that they are best separated. Some consider them as only representing different strains of the same organism. Some consider that the B. coli produces a bactericidal substance which inhibits the growth of, or destroys, pathogenic bacteria which may have passed the destructive influences of the gastric fluid; others that this effect is due to their free growth and the development of phenol and various putrefactive substances. The probable importance of the colon bacillus in protecting the organism is

shown by the fact that where numerous colonies of pathogenic organisms may be cultivated from fæces we may find a diminution in number or absence of the colon bacillus. This condition may be observed in infections with the organisms of dysentery, cholera, typhoid, and paratyphoid. While its normal function is probably protective, yet the B. coli is an important pathogenic agent, it being frequently the organism isolated from purulent conditions within the abdominal cavity, especially in appendicitis and lesions about the bile ducts. It is particularly prone to cause lesions of the bladder and pelvis of the kidney. In the treatment of colon cystitis by vaccines of dead colon bacilli, the most brilliant results in opsonic therapy have been obtained.

Sir A. Wright thinks that certain cases of mucous colitis may be due to colon infection and that vaccination may cure them. The colon bacillus is fully considered under the bacteriology of water.

B. cloacæ was isolated first from sewage by Jordan. It is a rapid liquefier of gelatin, and in its reactions with sugars and litmus milk resembles the colon bacillus.

Chromogenic Bacilli.

These are identified by the color of their colonies on agar. The B. pyocyaneus is the most important one of them in medicine, but the B. prodigiosus is also of interest medically. A violet chromogen, the B. violaceus, which is motile and liquefies gelatin, has been described under many names. It has been found in water.

An orange-yellow chromogen, the B. fulvus, is nonmotile and varies as to its liquefaction of gelatin.

B. pyocyaneus (Gessard, 1882).—This organism is frequently termed the bacillus of green or blue pus. It is a small (2.5 x .5μ) motile Gram negative bacillus. It grows readily at room or incubator temperature. It liquefies gelatin rapidly. The green color diffuses through the agar or gelatin on which it grows, so that we not only have the green-colored colony, but the medium as well is colored. Upon potato the colonies are more of a dirty brown. It is widely distributed in water and air, and is frequently isolated from fæces. The B. fluorescens liquefaciens of water seems to be simply a strain of B. pyocyaneus. The B. pyocyaneus is frequently associated with other pus organisms in abdominal abscesses. In addition to having an endotoxin, it produces

a soluble toxin similar to diphtheria toxin. This toxin differs from those of diphtheria and tetanus in that it can stand a temperature of 100° C., while those of diphtheria and tetanus are destroyed at about 65°C. The fact that the union between toxin and antitoxin is only of a binding, neutralizing nature is best shown by taking a mixture of pyocyaneus toxin and antitoxin which is innocuous and heating it. This destroys the antitoxin, but does not injure the toxin. We now find that the original toxicity has returned. The antitoxins of diphtheria and tetanus are more stable than the corresponding toxins; hence, this experiment would be impossible with them, as upon heating we should first destroy the toxin.

FIG. 35.—Bacillus pyocyaneus. (*Kolle and Wassermann.*)

B. prodigiosus.—This is a very small coccobacillus which shows motility in young bouillon cultures. It is Gram negative. The colonies on agar or other solid media show a rich red color. The pigment only develops at room temperature; it is absent in cultures taken out of the incubator. The B. prodigiosus is frequently found on food stuffs, especially bread, where it may simulate blood. It liquefies gelatin rapidly and gives a diffuse turbidity to bouillon. It is probable that B. indicus and B. kilensis are strains of B. prodigiosus.

Coley's fluid, which has been used in cases of inoperable sarcoma and other malignant growths, is a culture prepared by growing very virulent streptococci in bouillon for ten days. This streptococcus culture is now inoculated with B. prodigiosus, and after another ten

days the mixed culture is killed by heat at 60° C. and the sterile product injected. Coley injected about one-twentieth of a c.c. of this vaccine. At present he uses nonfiltered, heat sterilized bouillon cultures of a streptococcus obtained either from a case of erysipelas or septicæmia. To this is added material from agar cultures of B. prodigiosus, grown separately and sterilized before adding to the sterilized streptococcus bouillon culture.

CHAPTER IX.

STUDY AND IDENTIFICATION OF BACTERIA. SPIRILLA. KEY AND NOTES.

Key to recognition of gelatin-liquefying, motile and Gram-negative spiral or comma-shaped organisms.

A. **Do not give the nitroso-indol reaction** with sulphuric acid alone in twenty-four hours.
   1. Produce an abundant moist cream-colored growth on potato at room temperature.
      a. Finkler and Prior's spirillum. Liquefaction of gelatin very rapid. No air-bubble appearance at top of liquefied area. Cultures have foul odor. Milk coagulated. Thicker spirillum than cholera. Isolated from cholera nostras.
   2. Scanty growth or none at all on potato at room temperature. Only a moderate yellowish growth when incubated about incubator temperature.
      a. Spirillum tyrogenum. Does not liquefy gelatin so rapidly as Finkler Prior. Thinner and smaller spirillum than cholera.

B. **Give the nitroso-indol reaction** with sulphuric acid within twenty-four hours.
   1. Very pathogenic for pigeons.
      a. Spirillum metschnikovi. Liquefies gelatin about twice as rapidly as cholera. Gives bubble appearance at top of stab.
   2. Scarcely pathogenic for pigeons.
      a. Spirillum choleræ asiaticæ.

Nonmotile, nonliquefying and Gram-positive spirilla have also been described. There is also a large group of phosphorescent spirilla.

Spirillum Choleræ Asiaticæ (Koch, 1884).—Typically, the morphology of this organism is that of the comma (Comma bacillus of Koch). It also frequently shows S shapes, and often appears in long threads showing turns. When freshly isolated from cholera material they, as a rule, show a fairly typical morphology, but after subcultures in the laboratory variations are common, so that rod forms and round involution shapes give a picture altogether at variance with the comma shape. Even in recent cultures of undoubted cholera we may have different types, as coccoid forms and slender rods. Ohno has noted the fact that the same strain of cholera will give at one time vibrio forms and again coccoid or rod forms, depending on the reaction of the media. Inasmuch as the recognition of vibrio shapes is of importance in diag-

nosis he recommends that material from a stool be inoculated into three tubes of peptone solution of reaction +.3, −.5 and −1, respectively one of which would probably show vibrio morphology.

FIG. 36.—Cholera spirilla. (*Kolle and Wassermann.*)

The cholera spirillum is very motile (a scintillating motility) and liquefies gelatin fairly rapidly, although more slowly than any of the spirilla mentioned in the key. The colony on gelatin was formerly considered characteristic, but like most cultural characteristics, it is

FIG. 37.—Involution forms of the spirillum of cholera. (*Van Ermengem.*)

now considered as being only of confirmatory value; it is not specific. These colonies show in twenty-four hours as small granular white spots *which have a spinose* periphery. An encircling ring of liqefactuion now

makes its appearance and the highly refractile (as if fragments of sparkling glass) colony can be separated into a granular center, a striated periphery, and a clear external ring of liquefaction.

On gelatin stabs the liquefaction produces a turnip-like hollow at the top of the puncture—the air bubble appearance. It gives the nitroso-indol reaction with sulphuric acid alone (cholera red). Kraus attaches importance to the fact that cholera does not produce a hæmolytic ring on blood agar as do the pseudocholera spirilla; a difficulty is that many pseudospirilla do not hæmolize. It grows very rapidly on peptone solution and this is the medium for the enrichment test to be later described. On this it may form a pellicle. On agar the colony is more opalescent (more of a translucent grayish blue) than the typhoid. It does not grow on potato except at incubator temperature. It does not coagulate or turn acid litmus milk. The spirilla are found in myriads in the rice-water discharges, these white flakes being desquamated epithelial cells. They penetrate the crypts of Lieberkuhn, but rarely extend to the submucosa. The symptoms are due to an endotoxin.

FIG. 38.—Spirillum of cholera, stab culture in gelatin two days old. (*Fraenkel and Pfeiffer.*)

Cholera may be transmitted from water supplies, when the outbreak is apt to be widespread and in great numbers from the start. Also by indirect contagion, as by flies or on lettuce, etc. A very important point is that we have well persons whose fæces contain virulent cholera spirilla (cholera carriers).

Cholera spirilla disappear from the stools of cholera patients very rapidly, usually in two to three days. Cholera carriers are therefore of less importance epidemiologically than typhoid carriers. To identify such spirilla immunity reactions are necessary:

1. Injected intraperitoneally into guinea-pigs, it produces a peritonitis and subnormal temperature. This reaction exists for spirilla other than the true cholera spirillum.
2. Intramuscular injections into pigeons are only slightly pathogenic, if at all.
3. The agglutination test is the most practical. In this we use serum from an immunized animal, in dilution of from

100 to 1000. It is rare that true cholera vibrios fail to agglutinate in serum of 1 to 500 and even sera of 1 to 10,000 dilution give the reaction. Serum of cholera convalescents may show agglutination as early as the tenth day; it is usually best shown about the third week. Dunbar's quick method is very practical. Make two hanging-drop preparations, using mucus from the stool as the bacillary emulsion. To one add an equal amount of a 1:50 normal serum; to the other a 1:500 dilution of immune serum. Cholera spirilla remain motile in the control, but lose motility and become agglutinated in the preparation with the immune serum.

4. Pfeiffer's phenomenon. If cholera spirilla are introduced into the peritoneal cavity of immunized guinea-pigs (or if together with a 1:1000 dilution of immune serum the mixture is injected intraperitoneally into normal guinea-pigs) and at periods of ten to sixty minutes after injection, material is removed by a pipette from the peritoneal cavity, the spirilla have lost motility, have become granular and degenerated. Pseudospirilla are unchanged. This reaction may be carried on in a pipette, using fresh serum. Antisera for the treatment of cholera have not proved successful. Prophylactically, there are two prominent methods: (1) That of Haffkine, where live cholera spirilla are injected subcutaneously; and (2) Strong's cholera autolysate. In this cholera cultures are killed at 60° C. The killed culture is then allowed to digest itself in the incubator at 37° C. for three or four days (peptonization). The preparation is then filtered and from 2 to 5 c.c. of the filtrate is injected.

For diagnosis: 1. take a fleck of mucus, make a straight smear and fix; stain with a 1:10 carbol fuchsin. The comma-shaped organisms appear as fish swimming in a stream.

2. Inoculate a tube of peptone solution. The cholera spirilla grow so rapidly, and being strong aerobes, they grow on the surface of the fluid so that by taking a loopful from the surface, we may in three to eight hours obtain a pure culture. Inoculate a second tube from the surface of this first and, if necessary, a third (enrichment method).

3. *Test for* cholera red reaction. (Simply adding from three

to five drops of concentrated sulphuric acid in the first or second peptone culture after eighteen to twenty-four hour's growth.) At times we only get the cholera red when we have a pure culture of cholera.

4. Smear a fleck of mucus or, better, the three hour surface growth of a peptone culture on a dry agar surface in a Petri dish. From colonies developing, make agglutination and, if desired, cultural tests. It is by immune reactions that we identify cholera spirilla. The surface moisture of plates is best dried by the filter-paper top.

The cholera colony is easily distinguished from the ordinary fæcal bacterial colonies by its transparent, bluish-gray, delicate character. It emulsifies with the greatest ease. A practical, quick method is to make smears from suspicious colonies, stain for one minute with dilute carbol fuchsin and if vibrios are present to make two vaseline rings on a single slide allowing ample space at one end for handling the specimen safely. Inside of one ring deposit with a platinum loop a drop of salt solution and inside the ring nearest the end which is to be held by fingers or forceps, deposit a loopful of 1 to 500 or 1 to 1000 dilution of cholera serum. The emulsion in the salt solution remains uniformly turbid and under a low power of the microscope (2/3 in.) shows a scintillating motility. The emulsion made into the drop of serum quickly shows a curdy agglutination and upon examination with the 2/3-in. objective shows clumping and absence of motility. Cover-glasses placed over the two vaseline rings assist in the study of the preparation.

CHAPTER X.

STUDY AND IDENTIFICATION OF MOULDS.

Classification of the Fungi.

Order	Suborder	Family	Genus	Species
Phycomycetes	Zygomycetes		Mucor	M. corymbifer
				M. mucedo
Ascomycetes	Gymnoascus	Saccharomycetes	Saccharomyces	S. cerevisiæ
				S. anginæ
				S. blanchardi
			Endomyces	E. albicans
			Cryptococcus	C. gilchristi
				C. hominis
		Gymnoascea	Trichophyton	T. sabouraudi
				T. tonsurans
				T. violaceum
				T. mentagrophytes
				T. cruris
			Microsporum	M. audouini
			Achorion	A. schoenleini
	Carpoascus	Perisporiaceæ	Penicillium	P. crustaceum
			Aspergillus	A. fumigatus
				A. concentricus
				A. pictor
				A. niger
			Discomyces	D. bovis
				D. maduræ
			Madurella	M. mycetomi
Hyphomycetes			Malassezia	M. furfur
			Microsporoides	M. minutissimus
			Trichosporum	T. giganteum
			Sporotrichum	S. beurmanni

The Fungi.

The Thallophyta are plants in which there is no differentiation between root and stem.

The classes of Thallophyta which are of interest medically are 1. the Algæ and 2. the Fungi.

Some include Lichenes as a separate class. These are really symbiotic organisms—Fungi parasitic on Algæ.

The Algæ contain chlorophyll, with the exception of Cyanophyceæ.

To the order Cyanophyceæ it is considered that the family of bacteria belong.

The fungi do not possess chlorophyll. They are in their simplest forms ramifying filaments called hyphæ. The collection of vegetative hyphæ which intertwine in tangled threads, as a support, are termed the mycelium, while those which project upward are called the aërial hyphæ and are the ones which bear the conidia or spores.

The orders of the class fungi which are of interest medically are: 1. the Phycomycetes; 2. the Ascomycetes; 3. the Hyphomycetes.

Phycomycetes.—These produce a copious net-work like mycelium, which is nonseptate, and reproduce asexually by means of a sporangium, a case-like structure borne on the clubbed extremity of an erect hypha (columella) and containing numerous spores or, as in the case of the suborder Oomycetes, by heterogamy. (Dissimilar sexual cells—a smaller male, antheridium, and a larger female, oogonium. By fertilization by antherozoids from the antheridium penetrating the oösphere we have oöspores.)

The suborder Zygomycetes reproduces either asexually or by isogamy (two similar sexual cells conjugate and form on fusion a zygospore).

Belonging to this suborder are two species of pathogenic importance: 1. Mucor mucedo and 2. Mucor corymbifer. These moulds develop especially in external cavities as nasopharynx and external ear.

Pulmonary and generalized infections have also been reported. The pathogenic species have smaller spores and grow best at 37° C. The thick, coarse, cotton-like mould seen on horse manure is a Mucor. The sporangium, the organ of fructification, contains the spores within its interior. The M. mucedo has thick silver-gray mycelium, with large sporangia, 150μ in diameter, containing oval spores, 5 x 9μ. The M. corymbifer, which has been reported from a generalized infection, considered as typhoid, shows a snow-white mycelium. The sporangia are 20 to 40μ and the spore about 3μ in diameter.

Ascomycetes.—In this order are included many of the parasitic moulds. The most distinctive characteristic is the formation of ascospores in an ascus (little sac). It is an enlarged extremity of a hyphal branch in which a definite number of spores, usually eight, is formed. The ascus usually ruptures at its tip. Other members of the order are formed from hyphæ by the separation of cells in succession from the free cells. The mycelium is septate.

The order is divided into those with naked asci (Gymnoascus) and those having a perithecium or investing layer about the ascus (Carpoascus).

Belonging to the suborder Gymnoascus we have 1. the family of Saccharomycetes, which reproduce by budding and in which the asci are without any semblance of a sheath, and 2. a family in which there is an indication of the formation of a perithecium—this may be termed the Gymnoascea family.

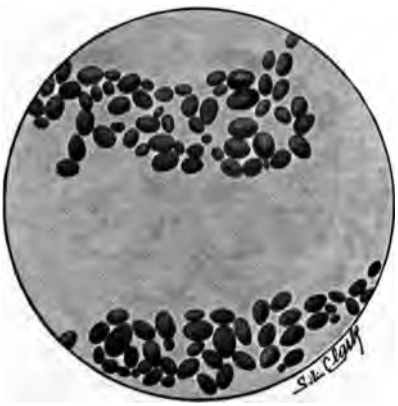

FIG. 39.—Yeast cells. Saccharomyces cerevisiæ. (*Coplin.*)

Saccharomycetes.—There are three genera: Saccharomyces, Endomyces, and Cryptococcus.

Saccharomyces.—These have ascopores.

S. cerevisiæ.—This is the ordinary yeast fungus. Used at times as an antiseptic.

S. anginæ.—Found in a case of angina.

S. blanchardi.—Found in a jelly-like tumor mass of the abdomen. The budding cells varied from 2 to 20μ. Probably identical with S. tumefaciens, reported as the cause of a subcutaneous tumor about region of Scarpa's triangle.

Endomyces.—Forms spores in the interior of filaments, or by ascus formation or by chlamydospores (resistant spore-like structures with a thick membrane which project from the extremities or sides of the hyphæ as bud-like structures).

E. albicans.—The organism of thrush. It produces a false membrane, especially on buccal surfaces, which is easily detached and beneath which the mucosa is intact. Grows only in acid media. Hence propriety of alkaline treatment.

Cryptococcus.—Reproduces by budding, but ascospore formation not observed. Not a well-recognized genus. The diseases caused by it are termed blastomycoses.

C. Gilchristi.—The cells are about 16μ in diameter and have a thick membrane. They reproduce by budding. The skin lesions resemble various infectious granulomata and diagnosis rests on the finding of budding or sporulating cells. It may invade internal organs. It is cultivable. A mould, somewhat similar, is the Coccidioides immitis of Ophuls. This has a mycelial

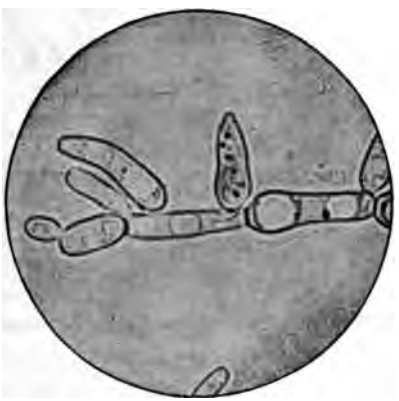

FIG. 40.—Thrush fungus. (*Kolle and Wassermann.*)

growth. The infection frequently becomes generalized. The small bodies, about 3μ, in the Molluscum contagiosum cells are thought by some to be yeasts. They are more probably artefacts. Plimmer's bodies in cancer cells belong in this group. They also are probably other than parasites.

C. Linguæ Pilosæ.—This is a more or less elongated yeast-like organism and supposed to be the cause of black tongue, a benign affection of the lingual papillæ.

Gymnoascea.—Belonging to the family Gymnoascea we have the genera Trichophyton, Microsporum and Achorion.

The trichophytons are generally known as the large-spored ringworms. The spores are in chains and may be inside the hair or both outside and inside. Many of them are of animal origin, especially from the horse and the cat. The spores are from 5 to 7μ.

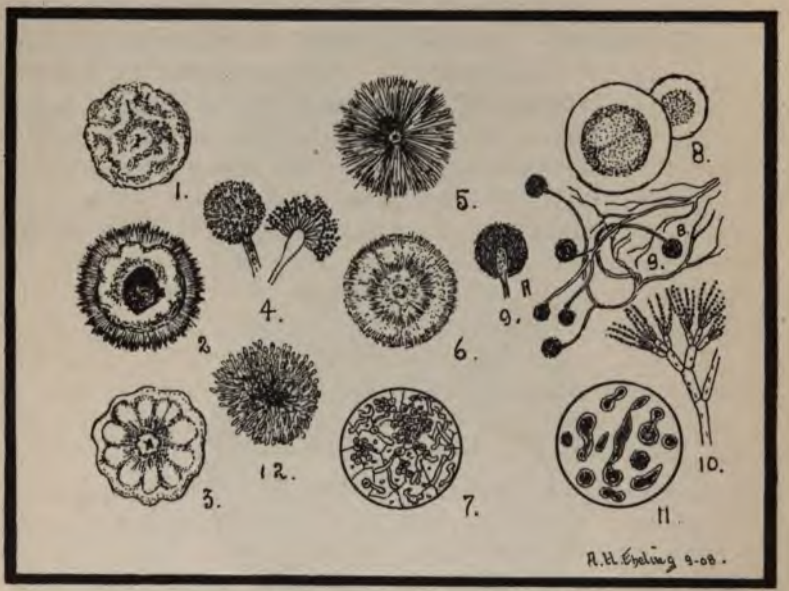

FIG. 41.—More common fungi. 1, Culture of Achorion schoenleini (favus); 2, culture of Trichophyton tonsurans; 3, culture of Trichophyton sabouraudi; 4, sporangium of Aspergillus; 5, culture of Trichophyton mentagrophytes; 6, culture of Microsporum audouini; 7, mycelium and spores of Malassezia furfur; 8, Cryptococcus gilchristi; 9, A and B, sporangium and mycelium of Mucor corymbifer; 10, Penicilium; 11, Saccharomyces tumefaciens; 12, Discomyces bovis.

The mycelium is greatly segmented, shows simple or dichotomous branching, and produces spores within the mycelium.

T. Tonsurans.—Gives a crater-like culture with fine marginal rays. Fungus wholly inside the hair. Causes most of the large-spored scalp ringworms and many body cases.

T. Sabouraudi.—Has a heaped-up festooned sort of culture. There is a similar fungus with a violet culture. These cause some of the scalp and beard ringworms.

T. Mentagrophytes.—This is the T. megalosporon endoectothrix of Sabouraud. The external spores are in chains or in short mycelial threads, not mosaics of spores. There are varieties from horse, cat, and bird. The lesions are more inflammatory than those of the endothrix class. Most of the beard and body ringworms belong to this group—very few scalp cases. The cultures are finely rayed.

Some give yellow cultures, others white and one derived from birds a rose-colored culture.

Microsporum audouini.—This is the so-called small-spored ringworm and is a very common and highly contagious affection of the scalp in children in England and France; less so in other countries. It almost exclusively affects the hairy scalp. The spores are 2 to 3μ in diameter. The broken stump of the hair is characteristic. The fungus is packed as a mosaic of spores, chiefly on the outside of the hairs. It gives a downy-white culture.

Achorion schoenleini is the cause of favus. The cultures are rather wrinkled. It is characterized by the scutulum or favus cup. This is a sulphur-yellow pea-sized cup with a central lusterless hair. Affected hairs may not show a cup. Favus is not so contagious as ringworm. It chiefly affects the hairy scalp, but may also invade the nails and even the body. Microscopical examination shows great irregularity of spores and mycelium, the latter being irregularly disposed and of varying thickness and length and wavy instead of straight as in Trichophyton. Cultures show ridges or convolutions.

In the suborder Carpoascus we have to consider the family Perisporiaceæ. In this family the asci are completely inclosed by the investing membrane, the perithecium. When this rots the spores are set free. There are two genera of interest, Penicillium and Aspergillus.

In Penicillium we have vertical branches with strings of conidia. In Aspergillus these conidia arise from a globular termination of the hypha.

Penicillium.—While Penicillium does at times form perithecia, yet

they characteristically show chains of spores. The common P. glaucum resembles a hand with terminal beads.

P. Crustaceum.—Is the common blue-green mould. It has been deemed pathogenic in cases of chronic catarrh of the Eustachian tube and in gastric hyperacidity.

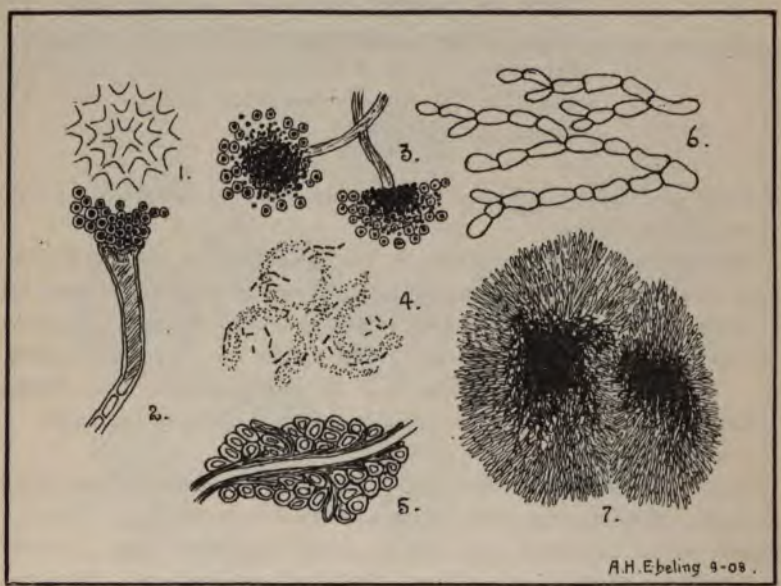

FIG. 42.—Tropical fungi. 1, concentric rings of Aspergillus concentricus; 2, sporangium of A. concentricus; 3, Aspergillus pictor; 4, Microsporoides minutissimus; 5, Trichosporum giganteum; 6, black granules of Madurella mycetomi; 7, yellow grains of Discomyces maduræ.

Aspergillus.—These have sterigmata carrying chains of spores. Of the pathogenic Aspergilli we have:

1. A. fumigatus.—This has been considered as the cause of pellagra. A pulmonary mycosis resembling phthisis may be due to this species.
2. A. repens.—This has been found in the auditory canal and may produce a false membrane.
3. A. flavus.—This has been found in the discharges of chronic ear diseases.

4. A. nidulans has been reported as one of the causes of mycetoma showing white granules. It has also been considered a cause of aural mycosis.

5. A. concentricus.—This is the cause of an important tropical ringworm, Tinea imbricata. The scales are dry, like pieces of tissue-paper. There are generally about four rings which do not heal in the center. General appearance is that of watered silk. There are no inflammatory lesions. Common in Malay peninsula. Also found in some parts of the Philippines and in China. Some authorities consider the fungus to be a Trichophyton.

6. A. pictor.—This is the cause of a skin affection of Central America. In the affection colored spots appear on the skin, chiefly on face, forearms, and chest. The disease is attended with a mangy odor. Spots are of various colors; if the superficial epithelium is affected we have a dark violet color. Deeper involvement gives red spots.

Hyphomycetes.—In this order are grouped certain genera which cannot properly be assigned to any of the other orders.

Discomyces bovis.—This is the well-known ray fungus, the cause of actinomycosis. In man it is at times found in chronic suppurative conditions attended with much granulation tissue. Such pus may show small yellow-gray granules about the size of a pin's head. When spread out between two slides the central portion shows a network of mycelium with bulbous thread-like rays going to the periphery. The "clubs" at the periphery are degenerate structures and do not stain by Gram. The central mycelium is Gram positive. This mould is essentially an anaerobe and should be cultivated in a deep glucose agar stab. It may also be cultivated in bouillon. In this it grows at bottom. Growth is dry and chalky. In diagnosis look for the little granules. Curetting of the sinuses may give the "ray fungus" when they are not found free in the pus.

Discomyces maduræ.—This is a ray fungus found in the yellow "fish-roe" granules of madura foot. It is strictly aerobic in cultures, thus differing from actinomycosis. For diagnosis proceed as for D. bovis.

Madurella mycetomi.—This is the cause of the black "gunpowder" granules of madura foot. It is a mycelial mass with rather oval shaped swollen segments. It is at times cultivable on potato and agar as felted masses of gray growth, which later becomes almost black.

Malassezia furfur.—This is the fungus of tinea versicolor. It is common both in temperate and in tropical climates. It is characterized by dirty yellow spots about covered parts of the body. Scrapings show a profusion of mycelial threads and interspersed spores. It is very difficult to cultivate. The organism usually termed the bottle bacillus is really a fungus having the characteristics of the genus Malassezia. It is thought to be the cause of pityriasis of the scalp.

Microsporoides minutissimus.—This is generally considered as the cause of Erythrasma or dhobie itch, a very common intertrigo of the tropics. It is characterized by its narrow mycelium and small spores. Various fungi are found in this affection. Castellani considers the cause of dhobie itch to be a trichophyton, T. cruris.

Trichosporum giganteum.—This is the cause of a disease of the hairs, known in Columbia as "Piedra," so called from the small gritty-like masses along the length of the hair. These spores are arranged like mosaics about the hair.

Sporotrichum beurmanni.—This fungus has a narrow mycelium (2μ) and branches in all directions. The spores appear as little grape-like clusters of oval spores (3 to 5μ) at the end of a filament. It is readily cultivated, showing, as a small white growth about the eighth day.

This mould produces indolent, glistening, subcutaneous tumors which are painless. They may ulcerate and give off a brownish discharge. They resemble tuberculous or syphilitic lesions.

Certain organisms which resemble both moulds and bacteria, having branching filamentous forms and at the same time having a spore-like method of reproduction, are known under the names Streptothrix or

better Nocardia. It is chiefly in various pathological processes of the lungs that they have been observed, but in addition they have been noted in brain, glands, kidney and subcutaneous tissue.

The infections are most likely to be confused with phthisis and glanders. The organisms are easily cultivated and in staining reactions are midway between T. B. and Actinomycosis.

Diagnosis of Fungi.

The most expeditious way to examine fungi is to treat the scales or hairs with a 10% solution of caustic potash or soda. Then crush between two slides; heat moderately over the flame and examine.

Tribondeau's method is to treat the scales with ether, then with alcohol, and finally with water. Next put the sediment (it is convenient to use a centrifuge) in a drop of caustic soda solution. Cover with a cover-glass, and after the preparation has stood about an hour run glycerine under the cover-glass.

A very satisfactory method is to scrape the scales with a small scalpel, and smear out the material so obtained in a loopful of white of egg or blood-serum on a glass slide. By scraping vigorously the serum may be obtained from the patient. After the smear has dried, treat it with alcohol and ether to get rid of the fat. It may then be stained with Wright's stain or by Gram's method. The ordinary Gram method may be used or the decolorizing may be done with aniline oil, observing the decolorization under the low power of the microscope.

Yeasts are best examined in hanging drop on the plain slide with vaseline cell, as given under Blood.

Cultivation of Fungi.

Moulds grow well on media with an acid reaction, so that by adjusting the reaction to +2% or even higher, we permit of the growth of the fungi, but inhibit bacterial development.

Glycerine agar, bread paste, or potato media are all suitable, but the best medium is that of Sabouraud:

Maltose,	4	grams.
Peptone,	1	gram.
Agar,	1.5	grams.
Water,	100	c.c.

Make the reaction about +2.

Before inoculating media with moulds, some recommend placing the material in 60% alcohol for one or two hours to kill the bacteria. The moulds withstand such treatment.

In cultivating moulds it is best to use small Erlenmeyer flasks, containing about one-fourth of an inch of media on the bottom, for the development of the colonies. In order to separate the mould we may take the hair or scales on a sterile slide and cut them into small fragments with a sterile knife. Then moisten a platinum loop from the surface of an agar slant, touch a fragment with the loop, and when it adheres transfer it to the agar slant. Make four or five inoculations on the surface and from suitable growth, after four to seven days, inoculate the medium in the Erlenmeyer flask.

Blauth recommends receiving the mould material between two sterile glass slides. Seal the edge of the slide with wax and place the preparation in a moist chamber for four to seven days. From developing fungus growth inoculate the medium in the Erlenmeyer flask. A Petri dish containing several layers of thoroughly moistened filter-paper in top and bottom makes a satisfactory moist chamber.

CHAPTER XI.

BACTERIOLOGY OF WATER, AIR, MILK, Etc.

Bacteriological Examination of Water.

While in a chemical examination as to the character of a water there are certain relations between the free and albuminoid ammonias, nitrates, nitrites, chlorides, etc., which indicate the probable animal as against vegetable nature of the organic matter present, yet it is a more or less presumptive evidence. In a bacteriological examination of water the finding of the colon bacillus may from a practical stand-point be considered as positive evidence of human fæcal contamination. Theoretically, the possibility of organisms being present corresponding culturally to B. coli and derived from cereals is to be considered. Also the fæces of animals contain an organism which cannot be differentiated from the colon bacillus.

In detecting sewage contamination in water to which varying amounts of sewage had been added, it was found that the bacterial tests were from ten to one hundred times more delicate than the chemical ones.

As showing sewage contamination of water, the presence of the B. coli has been generally accepted as the most satisfactory indication. The English authorities consider sewage streptococci and the spore-bearing B. enteritidis sporogenes as of value as indicators as well as the B. coli—the presence of sewage streptococci indicating very recent sewage contamination and that of the B. enteritidis sporogenes, in the absence of streptococci and colon bacilli, as evidence of sewage contamination at some period more or less remote.

In the United States the colon bacillus alone is considered the indicator of sewage contamination, and all tests, presumptive or positive, are based on the presence of this organism.

In collecting samples of water for bacteriological examination, the following points should be considered:

1. The bottles, which should have a capacity of from 25 to 100 c.c., should be sterile. Sterilization may be effected by heat or by rinsing with a little sulphuric acid and subsequently washing out thoroughly

with the suspected water before collection. The utmost care must be exercised that the fingers do not come in contact with the glass stopper of the neck of the bottle while filling it. If the specimen is to be sent some distance, it should be packed in ice to prevent bacterial development. Frankland states that a count of 1000 became 6000 in six hours and 48,000 in forty-eight hours. In water packed in ice for a considerable time, however, the bacterial count may diminish.

2. If collecting from city water supplies, secure the sample direct from the mains and let the water run from the tap a few minutes before collection. If the water be taken from a pond, stream, or cistern, be sure that the specimen comes from at least 10 inches below the surface. As sedimentation is the most important method in self-purification of rivers and ponds, it will be understood that any stirring up of the mud on the bottom will enormously increase a bacterial count.

Quantitative Bacteriological Examination.

1. Deliver definite quantities of the water to be examined into tubes of liquefied gelatin or agar and plate out the same in a series of Petri dishes.

A more practical method is to deliver the water from the graduated pipette into the empty sterile dish. The water should be deposited in the center of the plate and the melted gelatin or agar poured directly on the water and then, carefully tilting to and fro, mix the water and the media. One set of plates should be of gelatin and incubated at room temperature; a similar set should be of lactose litmus agar and incubated at 38° C. If the water is highly contaminated, it is necessary to dilute it; thus, with river water, which may contain from 2000 to 10,000 bacteria per c.c., a dilution of 1 to 100 would be desirable.

Ordinarily it will be sufficient to deliver from a sterile graduated pipette .2, .3, and .5 c.c. of the water in each of two sets of plates: one set for gelatin, the other for agar.

When gelatin is not at hand or convenient to work with, the gelatin plates may be replaced by others of lactose litmus agar for incubation at room temperature. After twenty-four hours at 38° C. or forty-eight hours at 20° C., the count should be made.

Example.—Forty colonies were counted on the gelatin plate containing .2 c.c. (1/5) of the water. The number of organisms would be 200 per c.c. Ten colonies were counted on the agar plate containing

.2 c.c. and incubated at 38° C. Number of bacteria developing at body temperature equals 50 per c.c.

There is no strict standard as to the number of bacteria a water should contain per c.c. Koch's standard of 100 colonies per c.c. is generally given. It is by the qualitative rather than the quantitative analysis that one should judge a water.

If there should be very many colonies on a plate, the surface can be marked off into segments with a blue pencil. If very numerous, cut out of a piece of paper a space equal to 1 square centimeter. By counting the number of colonies inclosed in this space at different parts of the plate, we can strike an average for each space of 1 square centimeter. To find the number of such spaces contained in the plate, multiply the square of the radius of the plate by 3.1416. Then multiply this number by the average per square centimeter, and we have the total number of colonies on the plate. This is the principle of the Jeffers disc.

The relative proportion between the bacterial count at 20° C. and that at 38° C. is of great importance from a qualitative stand-point, as will be seen later.

2. Deliver into a series of Durham fermentation tubes containing glucose bouillon and into another series containing lactose bouillon varying definite amounts of the water to be examined. In tubes showing the presence of gas in both glucose and lactose bouillon the evidence is presumptive that the colon bacillus is present. For the positive demonstration plates must be made from such tubes as show gas.

It is sufficient to deliver from graduated pipettes in each series quantities of water varying in amount from .1 c.c. to 10 c.c. In our laboratory we inoculate with .1 c.c., .2 c.c., .5 c.c., 1 c.c. and 10 c.c. of the suspected water. If the .1 c.c. tubes show gas, we have reason to assume that the water contained at least 10 colon bacilli per c.c. If only the 10 c.c. tubes showed gas—those with less amounts not having gas—we would be in a position to state that the water contained the colon bacillus in quantities of 10 c.c., but not in quantities of 1 c.c. or less. Many authorities regard water as suspicious only when the colon bacillus is present in quantities of 10 c.c. or less; waters of good qualities frequently showing the presence of the colon bacillus in quantities of 100 to 500 c.c.

It is generally accepted that if a water shows the presence of the colon bacillus in quantities of 1 c.c. or less, it should be regarded as suspicious.

At the present time the medium that gives the least source of error in carrying out the quantitative presumptive tests is the bile lactose. It is made by adding 1% of lactose to ox bile, and fermentation tubes of the media showing gas may be considered as very probably containing the colon bacillus. The percentage of error with this method is reported to be only 11%, while with glucose fermentation tubes the error is more than 50%. Gas formation is usually shown in forty-eight hours, but it is advisable to continue the incubation for seventy-two hours. Even with this method plates should be made.

3. As the colon and sewage streptococci ferment lactose with the production of acid and hence produce pink colonies on lactose litmus agar, much information can be obtained from the proportion existing between the number of pink colonies and those not having such a color. Waters of fair degree of purity rarely give any pink colonies.

Qualitative Bacteriological Examination.

General Considerations.—In some countries the proportion of liquefying to nonliquefying colonies on gelatin plates is considered of importance. Certain sewage organisms belonging to the proteus and cloaca group liquefy gelatin; consequently, if the proportion of liquefying to nonliquefying be greater than as 1 to 10, the water is considered suspicious. The test is not considered by American authorities as of any particular value.

The American Public Health Association recognizes the importance of the information obtained from a comparison of the number of organisms developing at 38° C. and those developing at 20° C. Bacteria whose normal habitat is the intestinal canal naturally develop well at body temperature, while normal water bacteria prefer the average temperature of the water in rivers and lakes. Consequently when the number of organisms developing at 38° C. at all approximates the number developing at 20° C., there is a strong suspicion that sewage organisms may be present. Normal waters give proportions of 1 to 25 or 1 to 50, while in sewage contaminated waters the proportion may be as 1 to 4 or less.

In addition, the appearance of pink colonies on the lactose litmus agar is of great assistance in judging of a water. Both sewage streptococci and the colon bacillus give pink colonies—those of the streptococci *smaller and* more vermilion in color. Microscopic examination

will differentiate the cocci from the bacilli. It is well to bear in mind that the pink colonies after twenty-four hours may turn blue in forty-eight hours from the development of ammonia and amines. Consequently the lactose litmus agar plates should be studied after twenty-four hours.

A good water supply will rarely show a pink colony, while in a sewage-contaminated one the pink colonies will probably predominate.

The diagnostic characteristics considered important by the American authorities in reporting the colon bacillus are:

1. Typical morphology, nonsporing bacillus, relatively small and often quite thick.

2. Motility in young broth cultures. (This is at times unsatisfactory, as some strains of the colon bacillus do not show it even in young bouillon cultures).

3. Gas formula in dextrose broth. Of about 50% of gas produced, one-third should be absorbed by a 2% solution of sodium hydrate (CO_2). The remaining gas is hydrogen. (Later views indicate that the gas formula is exceedingly variable and should not be depended upon. To carry out this test one fills the bulb of a fermentation tube with the caustic soda solution; then, holding the thumb over the opening or with a rubber stopper, the bouillon culture and the soda solution are mixed by tilting the fermentation tube to and fro. The total amount of gas is first recorded and then that remaining after the CO_2 has been absorbed is reported as hydrogen.)

4. Nonliquefaction of gelatin.

5. Fermentation of lactose with gas production.

6. Indol production.

7. Reduction of nitrates to nitrites.

NOTE.—The reduction of neutral red with a greenish-yellow fluorescence is very striking and has been suggested as a test for the colon bacillus. Many other organisms, especially those of the hog cholera group, have this power. It is convenient, however, to color glucose bouillon with about 1% of a 1/2% solution of neutral red.

Isolation of the Typhoid Bacillus from Water.

This is probably the most discouraging procedure which can be taken up in a laboratory. Only the most recent reports of such isolation from water supplies, which have been verified by immunity reactions,

can be accepted and of these the number of instances is exceedingly small. Owing to the long period of incubation, the typhoid organisms may have died out before the outbreak of an epidemic suggests the examination of the water supply.

There have been various methods proposed for the detection of the B. typhosus in water. A method which would offer about as reasonable a chance of success as any other would be to pass 2 or 3 liters of the water through a Berkefeld filter; then to take up in a small quantity of water all the bacteria held back by the filter. Then plate out on lactose litmus agar and examine colonies which do not show any pink coloration. The dysentery bacillus has about the same cultural characteristics as the typhoid one, so that it is important to note motility. If from such a colony you obtain an organism giving the cultural characteristics of B. typhosus, carry out agglutination and preferably bacteriolytic tests as well. Some strains of typhoid, especially when recently isolated from the body, do not show agglutination.

The Conradi Drigalski, the malachite-green, and various caffeine containing plating media have been highly recommended.

Isolation of the Cholera Spirillum from Water.

The method proposed by Koch in 1893 does not seem to have been improved upon by later investigators. To 100 c.c. of the suspected water add 1% of peptone and 1% of salt. Incubate at 38° C., and at intervals of eight, twelve, and eighteen hours examine microscopically loopfuls taken from the surface of the liquid in the flask. So soon as comma-shape organisms are observed, plate out on agar. The colonies showing morphologically characteristic organisms should be tested as to agglutination and bacteriolysis. Inasmuch as the true cholera spirillum shows a marked cholera-red reaction it is well to inoculate a tube of peptone solution from such a colony and add a drop of concentrated sulphuric acid after incubating for eighteen hours. The rose-pink coloration is given by the cholera spirillum with the acid alone—the nitroso factor in the reaction being produced by the organism.

Bacteriological Examination of Milk.

A bacterial milk count is of comparatively little value as showing whether a milk is dangerous or not. As a matter of fact, a milk which contains several million of bacteria per c.c. might be less dangerous

than one containing only a few thousand, especially if in the latter there were numerous liquefiers and gas producers present. There is, however, one point of importance in connection with the quantitative estimation of bacteria in milk, and that is the fact that in order to keep the development of the bacteria within the limits of 10,000 to 50,000 per c.c., it is necessary that the requirements of cleanliness in milking and the rapid cooling of the milk after obtaining it and the keeping of the temperature below 50° C. be rigidly observed. If a milk has a high count it shows some error in the handling of the milk. Anderson has found that top milk contains from ten to five hundred times as many bacteria as bottom milk. Centrifugally raised cream contains more bacteria than that forming by gravity. In making a quantitative bacteriological examination, the principle is the same as with water.

Make a known dilution of the milk with sterile water; add definite quantities of this diluted milk to tubes of melted agar or gelatin, and pour into plates. The diluted milk may also be delivered in the center of the plate and the melted agar or gelatin poured directly on it, mixing thoroughly. Always shake the bottle well before taking sample.

Example.—Added 1 c.c. of milk to 199 c.c. of sterile water in a large flask (500 to 1000 c.c.). After shaking thoroughly, take 1 c.c. of this 1 : 200 dilution and add it to 99 c.c. of sterile water. Shaking thoroughly, we have a dilution of 1 : 20,000. Of this we added .5 c.c. to a tube of gelatin or agar. After incubation the plate showed 75 colonies. Therefore the milk contained in each c.c. 75 x 2 x 20,000 (dilution) =3,000,000—the number of bacteria in each c.c. of milk.

Lactose litmus gelatin or agar is to be preferred in milk-work, as the normal lactic acid bacteria produce reddish colonies which are very striking. A standard easily attained for high-grade, certified milk would be 5,000 to 10,000 per c.c.

In the qualitative examination of milk, many dairies employ the fermentation tube, any organism producing gas being considered undesirable. Again liquefying organisms, as shown by the presence of such bacteria in the gelatin plates, is evidence of probable contamination by fæcal bacteria. A question which seems difficult to decide is as to the general nature of the so-called normal lactic acid bacteria of milk. Some describe them as very short, broad bacilli with very small colonies, fermenting lactose with the formation of lactic acid. Others consider that the streptococci are the organisms which are concerned with the

normal fermentative changes. In examining specimens of milk considered the best on the market, I have repeatedly found the small red colonies on lactose litmus agar to be streptococci. In connection with the organisms present in the tablets used for treating milk to produce lactic acid for the treatment of intestinal disorders, and considered to be normal lactic acid bacteria, I have found both streptococci and bacilli. These have all agreed, however, in not producing gas in either lactose or glucose fermentation tubes.

The organism upon which special stress is laid in these so-called lactic acid producers is the B. bulgaricus. This is a large, nonmotile organism with square ends like anthrax. It often occurs in long chains and does not possess spores. It is Gram positive. Colonies show in forty-eight hours which resemble streptococcus ones, but are more contoured on the surface. It produces a deep vivid pink in litmus milk, while milk streptococci only cause a light pink. It produces a very large amount of acid (3%). Little or no growth on ordinary laboratory media or below 37° C. (Op. temp. 42° C.).

Heinemann states that it occurs normally in human fæces and various fermented milks—also in gastric juice when HCl is absent. To isolate, put milk or fæces into a broth containing .5% acetic acid and 2% glucose. Transfer to litmus milk after twenty-four hours and from such tubes plate out on milk serum agar (coagulate boiling milk with a few drops of acetic acid, filter and add 1% peptone, 2% glucose and 1.5% agar).

Another source of information as to the quality of a milk may be derived from a study of the number of leukocytes or pus cells contained in 1 c.c. of the milk. It must be understood that cellular elements which differ only slightly from true pus cells may be found in the milk of healthy cows and may be found in great numbers. Statements have been made that such cells are neither amœboid nor phagocytic.

The Doane-Buckley method is probably the most accurate. In this you throw down the cellular contents of 10 c.c. of milk in a centrifuge revolving about 1000 times a minute for ten to twenty minutes. Then remove supernatant milk and add 0.5 c.c. of Toisson's solution to the sediment. You thus have the leukocytes of 10 c.c. contained in 0.5 c.c. (Concentrated twenty times.) Make a hæmatocytometer preparation as for blood and find the average number of cells for each square millimeter. Then multiply this by 10 to get the number of cells in a cubic millimeter. As a cubic millimeter is one thousand times smaller than a

cubic centimeter, you multiply the number per cubic millimeter by 1000. Then, as the milk was concentrated twenty times, you divide by 20. (If it were diluted twenty times, you would multiply by 20.)

Example.—Found an average of 50 cells per square millimeter. This would make 500 per cubic millimeter, and 500,000 per c.c.; then 500,000 divided by 20 would give 25,000.

There is no agreement as to a standard for allowable leukocytes. Even in apparently healthy animals they may exceed 100,000 per c.c. Doane has suggested 500,000 per c.c. as a preferable limit.

The smear methods for determining the number of leukocytes present do not compare in accuracy with the volumetric ones.

Bacteriological Examination of Air.

In Paris a cubic meter of air was found to contain the following number of organisms:

Suburbs.—	Winter,	145 moulds,	170 bacteria.
	Summer,	245 moulds,	345 bacteria.
City Hall.—	Winter,	1345 moulds,	4305 bacteria.
	Summer,	2500 moulds,	9845 bacteria.

Air of hospitals, especially after sweeping, may contain 50, 000 bacteria per cubic meter. There does not seem to be any particular relation between the amount of carbon dioxide in air and the bacterial content.

Petri's Rough Method.—Exposure of a lactose litmus agar plate (capacity 100 sq. cm.) for five minutes will give the number of organisms present in ten liters of air. Multiply by 100 for one cubic meter.

The two groups of organisms usually found in air are 1. bacteria and 2. moulds. Moulds (spores) may be carried by currents of air; bacteria, however, are generally carried about by particles of dust or finely divided liquids (spray). On the lactose litmus agar plate staphylococci and streptococci show as bright red colonies.

Sedgwick-Tucker Sterile Granulated Sugar Method.—Sterilize aerobioscope and introduce granulated sugar on support. Again sterilize (not over 120° C. in dry-air sterilizer). Allow a given quantity of air to pass through; then shake the sugar into wide part of aerobioscope. Now pour in 10 or 15 c.c. of melted gelatin (40° C.) to dissolve sugar. Roll tubes as for Esmarch roll cultures, and incubate at room temperature.

To draw air through the aerobioscope, connect the small end with a piece of rubber tubing which is attached to a tube in the stopper of an aspirating bottle. Having poured a definite quantity of water into the aspirating bottle, allow the water to run out. The same quantity of air will be drawn through the sugar of the aerobioscope as the amount of water passing out of the aspirating bottle. The bacteria and moulds are caught by the sugar.

Example.—Passed ten liters of air through the aerobioscope. The bacteria in this quantity of air showed seventy-five colonies when in-

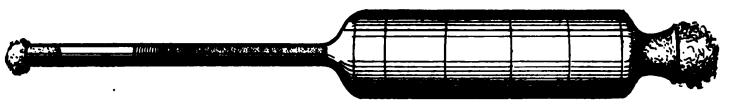

FIG. 43.—Sedgwick-Tucker aerobioscope. (*Williams.*)

cubated at 20° C. The unit being one cubic meter or one thousand liters, we have only obtained the bacteria of one hundredth of the unit. Hence multiplying 75 by 100 gives 7500 bacteria as present in one cubic meter of the air examined.

In comparing the results with the aerobioscope with those obtained by exposing a plate as in Petri's method for ten instead of five minutes, it was found that the latter was sufficiently in accord to make it a satisfactory approximate quantitative method. The simplicity and ease of access of the colonies developing in it make it preferable when the air of operating-rooms or hospital wards is to be examined.

CHAPTER XII.

PRACTICAL METHODS IN IMMUNITY.

THAT which prevents the gaining of a foothold by disease organisms in the animal body or which neutralizes their harmful products or destroys the parasites is termed immunity. In the main, the question of immunity hinges on the powers of resistance of the human body and the aggressiveness or virulence of the invading organism. It must always be kept in mind that immunity is only relative; thus the fowl, which is practically immune to tetanus, may be made to succumb by reducing its resistance by refrigeration or by increasing the amount of poison introduced. The insusceptibility which the fowl has to tetanus or which man has to many diseases of animals is best termed inherent immunity, and is at present only a subject of theoretical interest. When immunity to a given disease is obtained as a result of an attack of the disease in question or by laboratory methods of inoculation, this is termed properly an acquired immunity, and in the former case is a naturally acquired immunity or "natural immunity" and in the second is an artificially acquired immunity or "artificial immunity."

As a result of an attack of a disease or in response to the stimulus of the injection of the organism or its products, we have developed in the man so injected certain specific antagonistic properties to that organism, which are usually demonstrable in the blood-serum or other body fluids, and to which we apply the terms agglutinating power, opsonic power, or bacteriolytic power. The term antibody is also applied. All three powers may be present together in equal or in varying degree or one or more may be absent. By agglutinating power we mean that which causes evenly distributed organisms to come together and form clumps. By opsonic power we mean that which so alters the resistance of bacteria that the phagocytes ingest them. By bacteriolytic power we mean that which brings about disintegration or lysis of the specific organism. The bacterium which causes the disease or which is used in inoculation for the production of immunity is termed the specific organism.

Of the different kinds of immunity only artificial immunity will be considered. This may be obtained in two ways: 1. By injecting the bacteria or their products into man or animals and as the result of the activity of the cells of the animal invaded, antibodies are formed which neutralize the toxins of or destroy the specific bacteria. These antibodies which are supposed to be thrown off (free receptors) or which may remain attached to the cell (sessile receptors) may remain potential for months or years and so confer a more or less enduring immunity. This is termed active immunity. 2. When we take the serum of a man or animal immunized actively and inject it with its contained antibodies into a second animal or man, we confer an immunity on the second animal; but as his cells take no active part in the production of the immunity, but are only passive, we term this immunity "passive immunity." If this serum which is introduced in passive immunity only neutralizes the toxic products of the infecting bacteria, we term it antitoxic passive immunity and designate the immune serum as antitoxic serum. If it destroys the organism, we call it antimicrobic serum, and the immunity, antimicrobic passive immunity. Some immune sera are both antitoxic and antimicrobic.

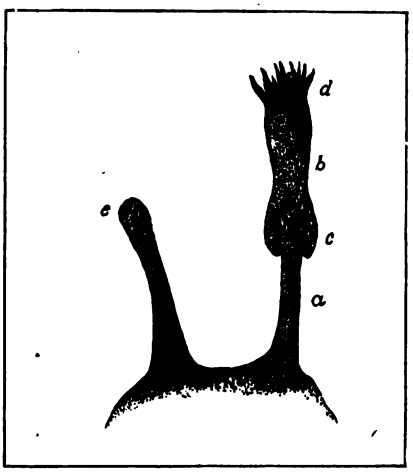

FIG. 44.—Receptors of the first order uniting with toxin. (*Journal of the American Medical Association*, 1905, p. 955.)
a, Cell receptor; *b*, toxin molecule; *c*, haptophore of the toxin molecule; *d*, toxophore of the toxin molecule; *e*, haptophore of the cell receptor.

It is well to remember that some organisms produce a toxin which is given off while the bacterium is alive; and in other instances the toxin is intracellular and is only given off when the bacterium disintegrates; consequently, an antimicrobic serum may cause the liberation of toxin. Diphtheria, tetanus, or botulism antisera are instances of antitoxic sera, while practically all others are antimicrobic. There is but one factor to consider in an antitoxic serum and that is the protoplasmic particles which are thrown off from the cell in response to the injury incident to

the attack upon the cell by the toxin particles. This free particle in the circulation represents the entire mechanism of antitoxic immunity. It is capable of uniting with the toxin molecule and neutralizing its toxic power, or rather so binding its combining end (haptophore group) that it is incapable of attaching itself to a cell, so that the poisonous end of the toxin (toxophore group) cannot have access to the cell. (For further discussion of toxins and antitoxins see under diphtheria, tetanus, botulism, and pyocyaneus infections.)

In antimicrobic sera we have two factors to consider, the first is a protoplasmic particle quite similar to the antitoxin molecule, but which in itself has no power of injuring its specific bacterium. This particle is generally referred to as the amboceptor or immune body. It is the specific product of the activity of a specific bacterium or foreign cell against the body cells attacked. It withstands a temperature above 56° C. and of itself is incapable of injuring the bacterium in response to whose attack it was produced. The second factor in the bacteriolysis of the specific bacterium, or the hæmolysis of the specific foreign cell, is something normally present in the serum of every animal, and which is capable of disintegrating a foreign cell or bacterium, provided it can have access to the cell or bacterium through an intermediary amboceptor (hence the amboceptor is sometimes called an intermediary body). This something is called the "complement." It is by some called "alexine," by others cytase (Metchnikoff). The complement cannot act upon and destroy an invading bacterium or cell unless the amboceptor is present to make the necessary connection. The complement is destroyed by a temperature of 56° C., so that, if we heat

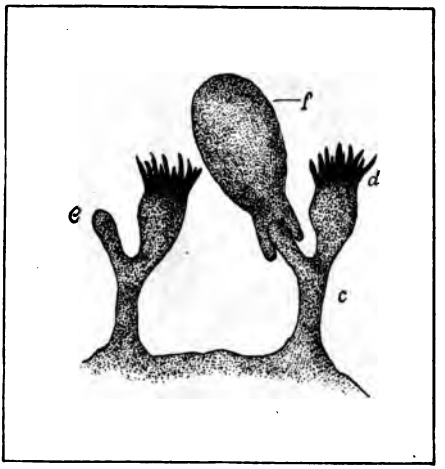

FIG. 45.—Receptors of the second order and of some substance uniting with one of them. (*Journal of the American Medical Association*, 1905, p. 1113.)

c, Cell receptor of the second order; *d*, toxophore or zymophore group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor.

the serum from an immune animal to 56° C., the complement it naturally contains is destroyed, and the amboceptor it contains, which is not injured by such a temperature, is incapable of destroying bac eria or cells, unless we replace the complement which has been destroyed by fresh complement. This is done experimentally by adding the serum of a nonimmunized animal which contains the complement, but no specific immune body (amboceptor) to the heated serum. This is termed "activating," and a serum so treated is said to be "activated." When

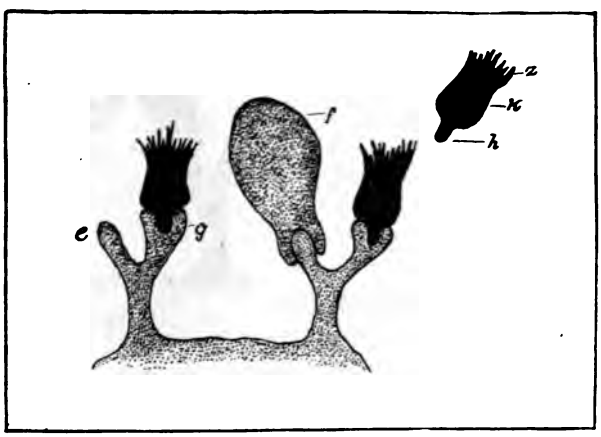

FIG. 46.—Receptor of the third order, and of some substance uniting with one of them. (*Journal of the American Medical Association*, 1905, p. 1369.)

c, Cell receptor of the third order—an amboceptor; *e*, one of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration (*f*) may unite; *g*, the other haptophore of the amboceptor with which complement may unite; *k*, complement; *h*, the haptophore; *z*, the zymotoxic group of complements.

an immune serum has been heated to 56° C., it is said to have been "inactivated."

When we allow a mixture of bacteria or cells to remain in contact with their specific immune serum which has been inactivated, the amboceptors attach themselves to the bacteria or cells, so that now, upon adding normal serum (complement), these bacteria or cells are so prepared that the complement can disintegrate them. This experiment *is termed* "sensitizing" and cells so treated are said to be "sensitized."

METHODS FOR OBTAINING IMMUNE SERA.

While a convalescent from a disease may be utilized to obtain an antitoxic, agglutinating, opsonic, or bacteriolytic serum against the specific bacterium, yet this is more conveniently obtained from an animal which has been immunized against the bacterium or cell in question. The

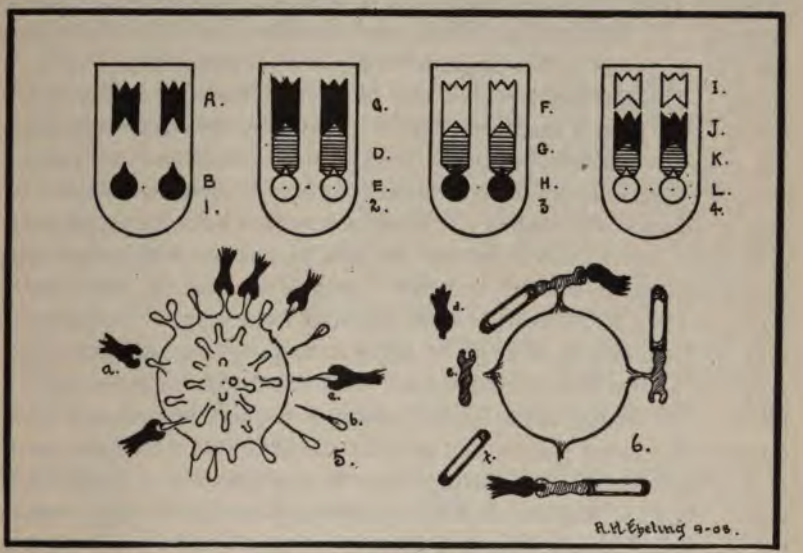

FIG. 47.—1, Red cells + normal serum. No amboceptor. No hæmolysis. A, Complement; B, normal red cell. 2, Red cells + immune serum. Complement and amboceptor. Hæmolysis. C, Complement; D, amboceptor; E, hæmolyzed red cell. 3, Red cells + immune serum heated to 56° C. Inactivated. Complement destroyed. No hæmolysis. F, Destroyed complement; G, amboceptor; H, red cells. 4, Red cells + heated immune serum + fresh serum. (Activated by contained complement.) Hæmolysis. I, Destroyed complement; J, fresh complement; K, amboceptor; L, hæmolyzed red cell. 5, Diagram showing antitoxin production. *a*, Toxin molecule; *b*, antitoxin molecule; *c*, neutralization of toxin by antitoxin. 6, Diagram showing bacteriolysin. *d*, Complement; *e*, amboceptor; *f*, bacillus.

rabbit is the most convenient animal to employ for the production of immune sera where the object is to have at hand a serum for use in diagnosis.

Where sera are used on an extensive scale, as in the production of curative sera, larger animals are employed. There are two applications of serum diagnosis: 1. Where the bacterium is known and the serum

is to be diagnosed. 2. Where the serum is known and the bacterium is to be diagnosed.

The first is employed by testing the agglutinating or bacteriolytic power of the serum taken from a patient upon pure cultures of the organism which is suspected as the cause of the disease. The Widal test (agglutination) is the best instance of this procedure. This method is of practical value in the diagnosis only of typhoid, Malta fever, and paratyphoid. In diseases like cholera and bacillary dysentery, the disease has run its course before agglutinating power becomes apparent in the serum. This method, however, may be used to prove that a convalescent has suffered from a suspected disease. Thus, by testing the agglutinating power of a serum, one or two weeks after recovery from a suspicious case of ptomaine poisoning, we may be able to demonstrate that the case in question was cholera. The second method has wider application, and is the one in which we use the sera of animals which have been immunized with known bacteria. Organisms isolated from urine, fæces, or blood of patients, or those obtained from water or food supplies may be identified by testing the agglutinating, opsonic, or bacteriolytic power of known sera against them. This has a wide range of applicability. The testing of the opsonic power of the sera in man or animals immunized against plague, and possibly cerebrospinal meningitis, seems to give more definite information than do agglutination or bacteriolytic tests. With the majority of other organisms, however, the agglutination test is the one almost always preferred.

Even in a small laboratory there are no particular difficulties in the way of having on hand rabbits immunized against typhoid, paratyphoid, Malta fever, acid-producing and nonacid-producing strains of dysentery, cholera, etc. Just as we inject men with vaccines prepared from various bacteria in opsonic therapy, so we inject animals to produce sera for diagnosis. We may use either a bouillon culture or the growth on agar slants taken up with salt solution as the inoculating material. This is heated for one hour at 60° C. to kill the bacteria. Where we desire to produce a serum which will disintegrate red blood cells (hæmolytic serum), we inject about 4 c.c. of the defibrinated blood of the animal for which we wish to produce a specific serum. Thus for a serum for use in a medicolegal case we would inject the rabbit with human blood. The most convenient way to defibrinate blood is to break a section of glass tubing into fragments, put these fragments into a glass test-tube,

sterilize tube and contents in a flame or sterilizer and, when cool, let the blood drop into the test-tube (we may use a Wright's pipette with a rubber bulb to take up the blood from a puncture of the finger and eject it into the tube). By shaking, the fibrin collects on the glass fragments, and we have the corpuscular emulsion to inject. Inject about 4 c.c. of the defibrinated blood or 1 c.c. of the killed bacterial bouillon culture into the peritoneal cavity of the rabbit. The easiest way to inject the rabbit is to hold the animal head down and plunge the needle in the median line into the abdominal cavity, forcing in the contents of the syringe. The intestines gravitate downward and by entering the needle below the limits of the bladder we avoid injuring any vital part. It may be more satisfactory to at first inject only about 1/2 c.c., and then if there is very little reaction, as shown by the appetite and spirits of the rabbit, to inject about four days later 1 c.c. About four or five injections at intervals of three to five days will usually produce an immune serum. Some animals do not seem to be capable of producing antibodies, so that it may be necessary to use one or more rabbits before a satisfactory serum is obtained. The most convenient way of obtaining serum for a test is to cut across one of the marginal veins of the rabbit's ear, and collect the blood in a Wright's U-tube. Centrifugalizing, we have the serum ready for use.

The vein can be made to stand out prominently by applying a compress dipped into very hot water. When a large amount of serum is desired it is better to use a test-tube with two pieces of glass tubing passing through a double perforated rubber stopper. To one of the projecting pieces of glass tubing a stout hypodermic needle is attached through the medium of 8 inches of rubber tubing and to the second piece of glass tubing passing through the stopper of the large test-tube another piece of rubber tubing is attached for suction. To obtain blood from the rabbit find the ensiform cartilage and insert the needle in the notch to the left and gently force it upward. Applying suction with the mouth the blood flows into the test-tube so soon as the needle enters the heart. By placing the tube of blood in the refrigerator the serum separates out from the clot. The removal of 20 to 30 c.c. of blood does not seem to affect the animals in the least and they can be used in this way time and time again. The immune body and agglutinin in serum remain active for weeks when kept in the refrigerator. The complement and opsonin, however, begin to deteriorate at once and have disappeared

by the fifth day. Consequently, for opsonic and bacteriolytic and hæmolytic experiments, fresh serum—twelve to twenty-four hours—must be used, or it may be activated.

Agglutination Tests.

There are two methods of testing the agglutinating powers of a serum—the microscopical and the macroscopical or sedimentation method.

1. For the microscopical method draw up serum to the mark .5 of the white pipette. Then draw up salt solution to the mark 11. This when mixed gives a dilution of 1 to 20. One loopful of the diluted serum and one loopful of a bouillon culture or salt solution suspension of the organism to be tested gives a dilution of 1 to 40. One loopful of the 1–20 diluted serum and 3 loopfuls of the bacterial suspension give a dilution of 1–80. These two dilutions answer in ordinary diagnostic tests. The red pipette with a 1–100 or 1–200 dilution may be used where dilutions approaching 1–1000 are desired. Having mixed the diluted serum and the bacterial suspension on a cover-glass, we invert it over a vaselined concave slide and examine with a high power, a dry objective (1/6 in.). It is simpler to make a ring of vaseline to fit the cover-glass and make the mixture of diluted serum and culture in the center of this ring or square. Then apply the cover-glass, press it down on the vaseline ring and examine as with the ordinary hanging drop. In making dilutions it is preferable to use salt solution, as the phenomenon of agglutination requires the presence of salts. Ordinarily, thirty minutes is a sufficient time to wait before reporting the absence of agglutination. Agglutination is more rapid at body temperature than at room temperature. In reporting agglutination, always give time and dilution. It is absolutely necessary that a control preparation be prepared in every instance; that is, one with the bacterial culture alone or with a normal serum of the same dilution as the lowest used. Some normal sera will agglutinate in 1 to 10 dilution, and group agglutinations (as paratyphoid with typhoid serum) may occur in 1 to 40 or possibly higher. It is very unusual for sera to agglutinate any other bacteria than the specific one in dilutions as high as 1–80.

2. For the macroscopical or sedimentation test, take a series of small test-tubes (3/8 x 3 in.) and deposit 1 c.c. of salt solution in each

of the series. Now, having taken an empty test-tube, drop 4 drops of serum in it and then add 12 drops of salt solution. This approximately gives 1 c.c. of a 1–4 dilution of the serum. With a rubber-bulb capillary pipette, which has been graduated to hold 16 drops or 1 c.c. draw up the contents of the tube containing the 1 to 4 serum and add it to the next tube containing 1 c.c. of salt solution. This gives a dilution of 1 to 8. Now mix thoroughly by drawing up and forcing out with the bulb pipette, and then withdraw 1 c.c. and add to the next tube containing 1 c.c. of salt solution. This gives a dilution of 1 to 16. Having mixed as before, again withdraw 1 c.c. of the mixture and add it to the 1 c.c. in the next tube. We now have a dilution of 1 to 32. Again withdrawing 1 c.c. and adding it to the fourth tube containing 1 c.c. of salt solution we have a dilution of 1 to 64. In tube 1 there is 1 c.c. of a dilution of the serum of 1 to 8; in tube 2, there is 1 c.c. of a dilution of 1 to 16; in tube 3 of 1 to 32. Tube 4 contains 2 c.c. of 1 to 64. Now adding 1 c.c. of a culture of typhoid or any other organism, we have the dilution of the serum in each tube doubled. Tube 1 now contains a serum in dilution of 1 to 16, acting on the bacteria; tube 2 of a 1 to 32; tube 3 of a 1 to 64. Now place these tubes in the incubator and, after two to five hours or overnight, we examine for the clearing up of the supernatant fluid. If the serum in a certain dilution agglutinates, the clumps gravitate to the bottom and the upper part becomes clear. If so desired, these dilutions may be carried on to 1 to several hundred in the same way. It is safer to work with dead cultures instead of living ones. To prepare, take a twenty-four-hour agar slant culture of typhoid or paratyphoid and emulsify in salt solution (about 6 c.c. to a slant).

By adding .1 of 1% of formalin to the typhoid emulsion and placing in the ice-box the cultures will be found sterile in about three days. The emulsion should be shaken twice daily while undergoing sterilization in the ice-box. Such cultures are not easily contaminated and appear to retain their agglutinable qualities for several months. The macroscopic methods are preferable with such dead cultures.

A very convenient method in general use in Germany is the following: Make dilutions of serum in ordinary test-tubes (3/4 x 6 in.) as described for the small test-tubes. Then take a loopful (2 mg.) of culture from an eighteen to twenty-four-hour-old agar culture and emulsify it thoroughly in the dilution in the first test-tube—repeat the process in the second tube and so on. This procedure is much safer than when live cultures

are added with a pipette. Again, the dilution is unchanged by this addition whereas it is doubled when an equal volume of culture is added to the diluted serum. A control should always be made in normal salt solution. After incubating, observe flocculent precipitates (agglutination) by tilting the fluid in the tubes to form a thin layer and to obtain the most advantageous light and look for a fine curdy precipitate (agglutination) or a uniformly turbid emulsion (negative reaction).

The method of using a slide with two vaselined rings, one containing an emulsion in the specific serum and the other in salt solution is of great practical value. This method is described under cholera.

Pfaundler under the designation of a thread reaction showed that organisms tended to grow in thread forms in a culture medium containing the homologous serum. Mandelbaum has suggested this as a means of diagnosing typhoid. Take ordinary bouillon containing 1% of sodium citrate. Inoculate it with a culture of typhoid. Now with a bulb capillary pipette take up one part (as marked by a wax pencil) of the patient's blood and fifteen times as much of the citrated bouillon just inoculated with typhoid. Mix the blood and citrated bouillon on a sterile slide or in a test-tube and after drawing up into the lower part of the expansion of the capillary pipette, seal off the capillary end. Now place the sealed-off pipette upright in an incubator and after four or five hours take out from the expanded end a loopful of the clear supernatant fluid (the blood cells settle to the bottom) and if the typhoid bacilli are in chains instead of being single and motile it shows a positive reaction.

Hæmolytic Experiments.

Take the blood of the animal that has been used to immunize the rabbit and receive it in a graduated centrifuge tube containing salt solution which has had 1% of sodium citrate added to it. This prevents the coagulation of the blood. After mixing, centrifuge and pipette off supernatant fluid. Note the graduation reached by the sediment of red cells and make up with salt solution to twenty times its volume. If the cells reach the 1/2 c.c. mark, add 10 c.c. of salt solution. This gives a 5%e mulsion of red cells—the percentage usually used in hæmolytic experiments. To carry out the test, simply add 1 c.c. of this 5% mixture of red cells to each of the series of tubes containing diluted serum,

as with the macroscopic agglutination tests. Place in the incubator for two to five hours. The red cells settle to the bottom, and tubes showing hæmolysis have a light reddish to rich hæmoglobin color. Tubes not showing hæmolysis remain white.

Bacteriolytic Experiments.

These may be carried out in the peritoneal cavity of a guinea-pig, injecting mixtures of immune sera and the bacterial culture.

Upon withdrawing, after fifteen to sixty minutes, the bacteria are granular and disintegrated. This is the well-known Pfeiffer's phenomenon, and was once considered the most important test for cholera. See Cholera. There have been several accidents (death of Orgel) with this test, and it is not practicable except in a well-equipped laboratory. Instead of a guinea-pig, we may simply take a fresh serum of known dilution, and mix it with an equal quantity of the bacterial emulsion in a capillary pipette; sealing off the end of the pipette, we incubate for 15 minutes. Then filing off the end we mix the culture thoroughly on a sterile slide; deposit a small drop for a hanging-drop preparation and draw up the remaining mixture into the same tube and again incubate. The time and dilution with which the culture becomes nonmotile and granular (bacteriolytic disintegration) should be recorded. Controls with normal serum are always necessary.

Deviation of the Complement.

It has been found that if there is not sufficient immune body in a mixure of normal serum, containing abundant complement, and bacterial emulsion, only a portion of the bacteria present will be destroyed. Increasing the amount of immune body with a constant quantity of normal serum, we reach a point where all the bacteria are destroyed. Now, if we continue to increase beyond this point the addition of immune serum, the destruction of the bacteria ceases, and the cultures will again contain myriads of living bacteria.

To carry out the test, make a series of tubes containing mixtures of bacteria with the same quantity in each of normal serum. Thus, each tube contains 1/2 c.c. of bacterial emulsion and 1/2 c.c. of 1–10 normal serum. Now inactivate a tube of 1–100 immune serum and to each of

the tubes of normal serum and bacterial emulsion add increasing drops of the inactivated 1–100 immune serum. Thus, 1 drop to No. 1 tube; 2 drops to No. 2 tube, and so on. After incubating for two hours, we take a pipette and plate out a fraction of a drop in an agar plate. The limit at which bacteriolysis is complete is shown by there being an absence of colonies.

Beyond or below that point colonies are more or less abundant. The explanation of this phenomenon of deviation or deflection of the complement is that where we have an excess of amboceptors for available receptors on the bacterial cells, only a portion of the amboceptors can attach themselves to their specific bacteria. The free amboceptors, not being able to form a union with the bacterial cell receptors (for which they have a greater affinity), combine with the complement present. Unless the complement be in excess, there will be no free complement left to join onto the amboceptors attached to the bacterial cells, and consequently bacteriolysis does not take place and the plate cultures show an abundance of colonies.

Fixation or Absorption of the Complement.

One of the controversies in connection with the nature of the complement is that regarding the question of the unity of complements or whether there exist different kinds of complements for different amboceptors (unity and multiplicity of complement). To prove that a single complement will act with varying amboceptors, Bordet and Gengou showed that the same complement would activate both hæmolytic and bacteriolytic immune bodies. If to a mixture of typhoid bacteria and inactivated typhoid immune serum some guinea-pig serum is added and the mixture be allowed to remain at 37° C. for two hours, and then sensitized red cells be added and the mixture again be placed in the incubator for two hours, no hæmolysis will be found to have occurred, because the bacteria have absorbed all the guinea-pig complement through the intervening typhoid amboceptors, and there is no complement left to hæmolyze the red cells through the specific blood-cell amboceptors. If, instead of immune typhoid serum, the serum of a normal person had been used, there would have been no amboceptors to unite the complement to the bacterial cells. The complement would then be at hand to unite with the sensitized red cells subsequently added and

bring about their hæmolysis, as shown by the ruby color of the supernatant fluid. This phenomenon of Bordet and Gengou has been utilized by Wassermann for the diagnosis of diseases where cultures are not applicable. It is in the diagnosis of syphilis that it is best known. It at present being impracticable to obtain cultures of Treponema pallidum, we use an emulsion of the liver of a syphilitic fœtus, which has been filtered so as to be clear, instead of a culture. The syphilitic liver, as can be observed by staining according to Levaditi's method, is packed with spirochætes.

It has now been found that lecithin or, preferably, emulsions of various normal organs may be substituted as antigen for the syphilitic liver, the antigenic power being due to lipoids. Aqueous extracts contain in addition to lipoids, substances which render the antigen unstable—alcoholic extracts are more stable and contain less anticomplement.

For the immune bodies we take the serum of the patient, or if a case of locomotor ataxia or general paresis, the cerebrospinal fluid. This may be heated to 56° C. to destroy complement (inactivation).

The following is a modification of Noguchi's method:

For the antigen dissolve 0.3 gm. lecithin in 50 c.c. of absolute alcohol, now add 50 c.c. of normal salt solution; shake well and filter until the filtrate is perfectly clear.

As some specimens of lecithin are unsatisfactory it is better to take 15 gm. of normal guinea-pig liver or kidney and emulsify in 150 c.c. absolute alcohol. Allow the mixture to stand for several days in the incubator (37° C.) then filter and concentrate to 50 c.c.

For the immune serum use that of rabbits which have received five or six injections of washed human red cells intraperitoneally at intervals of five days. (From 5 to 20 c.c. at each injection.) This immune serum should preferably be capable of dissolving human red cells in a dilution of 1–1000 (hæmolysis).

For the suspension of red cells add 1 drop of the blood of a normal person to 4 c.c. of salt solution.

For complement use fresh guinea-pig serum in a dilution of 1 to 25.

Experiment.—Take four small test-tubes (3/8 x 3 in.), label 1a, 1b and 2a, 2b, respectively. Into 1a and 1b each put 1 drop of the serum of the patient to be tested and into 2a and 2b each put 1 drop of the serum of a person known to give a positive test for syphilis. Next add to each of all four tubes 1 c.c. of the suspension of red cells (1/4 drop in each). Then add to each tube 1 c.c. of the 1–25 fresh guinea-

pig serum. Now add to tube 1a and tube 2a each 1 drop of the solution of antigen—tubes 1b and 2b are controls not containing antigen. Mix contents of tubes thoroughly and incubate at 37° C. for one hour. Now add to each of the four tubes 2 units of the immune hæmolytic serum (1 unit is sufficient immune serum to hæmolize the quantity of red cells, 1/4 drop, in the presence of 1 c.c. of 1–25 fresh guinea-pig serum; hence with a serum having a titre of 1 to 1000, 1/10 c.c. of a 1–100 dilution would represent one unit). After shaking thoroughly incubate the tubes again at 37° C. for two hours.

The tubes without antigen (1b and 2b) should show good hemolysis. Tube 2a, that of the known syphilitic, with antigen, should not show hæmolysis and that of the person examined (1a) should show hæmolysis in case the test is negative for syphilis. Moderately positive cases may show a slight trace of hæmolysis. In case the tubes without antigen are negative (no hæmolysis), repeat the test with smaller amounts of human serum. It may be advisable to employ the serum of a person known to be free from syphilis. In this case we should use two additional tubes, 3a and 3b, conducting the test as for the syphilitic control serum.

In the Wassermann reaction the rabbits are injected with sheep red cells which have been washed twice with salt solution by aid of the centrifuge. About five injections with, on the average, the quantity of red cells contained in 5 c.c. of sheep blood given at intervals of five days gives a strong hæmolytic serum if taken about one week after the last injection. The method is to take in a test-tube .2 c.c. inactivated human serum (heated for one hour at 56° C.), .1 c.c. fresh serum from guinea-pig for complement, 1 unit antigen and 3 c.c. normal salt solution; then to incubate for one hour at 37° C. (An antigen unit is the amount that will inhibit hæmolysis of 1 c.c. of 5% emulsion of sheep cells when mixed with .2 c.c. luetic serum and .1 c.c. guinea-pig complement.) Then add 2 units of amboceptor and 1 c.c. 5% emulsion of sheep red cells, shake and incubate for one hour. (The amount of hæmolytic serum that will hæmolize 1 c.c. of a 5% emulsion of sheep red cells to which .1 c.c. guinea-pig serum has been added in one hour is an amboceptor unit.)

The same technic is employed with the control test-tube except that the antigen unit is not put in.

The Noguchi method gives a positive reaction with nonsyphilitic sera in about 7% of cases. The Wassermann gives a negative result in about

9% of syphilitic sera. These figures show the advantage of checking one against the other.

In 268 cases at the medical clinic of Johns Hopkins Hospital, Clough failed to obtain a positive reaction in ninety-nine cases which were negative clinically.

In forty-five cases of syphilis he obtained 73% of positive results. Excluding cases which had received thorough treatment 82% were positive. Tabes gave 40% and general paresis 100%. In five cases of primary syphilis four gave positive reactions.

Based upon the observation of Bauer, that human serum contains hæmolytic amboceptors for sheep corpuscles, and of Hecht, that the complement normally present in human serum would suffice without the addition of guinea-pig serum complement, the following method of Fleming is easy of application.

For the test we use:

1. Alcoholic extract of rabbit's heart, made by washing the recently removed heart with salt solution to remove all blood. Cut into small pieces and grind in a mortar with sand and for every gram of heart add 5 c.c. of 95% alcohol. Keep the mixture at a temperature of 60° C. for two hours and filter. This is the stock solution. For use dilute it ten times with normal salt solution.

2. A 5% emulsion of washed sheep red cells, prepared as for the Wassermann test.

3. Suspected and control sera.

With a capillary bulb pipette take up one part of serum and four parts of the heart antigen, mix on a glass slide, again draw up into the capillary pipette and, leaving a separating air space, next draw up one part of 5% emulsion of sheep red cells. Then seal off tip of pipette and incubate at 37° C. for one hour. Now file off tip and mix the red cells with the serum and antigen and again draw up into the capillary pipette and incubate a second time for two hours. Hæmolysis or the reverse is shown in the fluid overlying the cell sediment. Various controls should be made using normal and known syphilitic sera; also with normal salt instead of serum.

The objections to methods using human serum for complement are 1. the great variation in the complement content of different human sera; 2. human complement requires about ten times as much amboceptor as guinea-pig complement and is less sensitive to fixation.

In connection with the Noguchi test, filter-paper impregnated with hæmolytic serum and dried may be used. Antigen paper is also practicable. These dried papers make the test practicable outside of a large laboratory.

Where antigen is prepared from animal organs the human sera to be tested should be inactivated (heated to 56° C. for twenty minutes) for the Noguchi test.

The various precipitate tests that have been proposed are unreliable. The precipitate reactions with bile salts give better results than with lecithin, this latter showing positive results in almost one-half of non-syphilitic cases.

Determination of Opsonic Power and the Preparation of Vaccines.

The following modification of Leishman's method takes very little time and skill and is applicable in the determination of the organism concerned in an infection, as in Wright's method. The control of vaccine treatment by taking opsonic indices from time to time does not seem to have met with much favor in this country—the sources of error being as great, if not greater, than ordinary variations in the opsonic index during the negative and positive phases. *Method:* Emulsify, by repeatedly drawing up and ejecting with a bulb capillary pipette, a small loopful of a young agar culture of the organism to be diagnosed in 12 to 15 drops of salt solution containing 1% of sodium citrate (the citrate prevents coagulation). This may most conveniently be done in a watch-glass. Now puncture the ear of the patient and draw up blood to a point marked on the capillary bulb pipette with a grease pencil. Draw in air to make a slight break in the column and then draw up bacterial emulsion to the same mark. Thoroughly mix the blood and emulsion on a slide by drawing up and ejecting the mixture. Then finally draw up the mixture to about 2 inches above the tip and seal the tip off in the flame. Put the pipette preparation in the incubator for exactly fifteen minutes. Prepare a similar preparation, using the blood of a normal person. At the expiration of fifteen minutes' incubation for the patient's blood and the same time for the control blood, file off the sealed tip, cautiously eject the contents on a slide, and after mixing, spread a film on a slide or cover-glass. Fix with burning

alcohol and stain with formol fuchsin. With Wright's stain the dark nucleus obscures some of the bacteria. Counting the phagocytized bacteria in a given number of polymorphonuclears, we obtain an average number of bacteria phagocytized per cell. Repeating the count with the control or normal blood, we likewise have the average number of bacteria taken up per cell. Dividing the patient's average by the normal average, we have the opsonic index. If the average for fifty of the patient's cells was eight and that of the control only four, the patient's index would be two, or twice the normal. The practical value of this test is where two or more organisms are in a body fluid we may ascertain the causative organism by noting marked variation from the normal in the patient's opsonic index for that particular organism and not for the other organism. This variation may be of the nature of a high or low opsonic index.

Preparation of Vaccines.—It has been found satisfactory to make use of stock vaccines in gonorrhœal and tuberculous affections. In case of other infections, however, and preferably with gonorrhœal infections, the causative organism should be isolated from pus, sputum, urine, blood, or other material (autogenous vaccine). In treatment of tuberculosis Wright prefers Koch's T. R. or Neu Tuberculin in doses of from 1/5000 to 1/800 of a milligram. Some prefer Koch's more recent Bazillen emulsion. Having isolated the organism, it is inoculated upon one or more agar slants, and after a growth of from five to seven hours with streptococci and pneumococci, or with eighteen hours for staphylococci and colon, the growth on these inoculated slants is taken up with salt solution, thoroughly shaken up in the diluting solution and standardized.

The most practical way is to gently rub off the growth on the agar in about 1 or 2 c.c. of salt solution with a platinum loop. Then pour the bacterial emulsion into a sterile test-tube and repeat the process with three to five agar slants, until we have from six to 10 c.c. of the emulsion in the sterile test-tube. By heating to melting-point in the flame a piece of glass tubing and attaching it to the rim of the test-tube (also melted), we have a handle with which to draw out the test-tube when heated about 1 inch from the mouth in a blowpipe flame. Drawing this out, we let it cool, and then filing the constricted portion we break it off and seal it in the flame. By shaking up and down vigorously for five to fifteen minutes, the bacteria are distributed evenly in the salt

solution. The sealed test-tube is then placed in a water-bath at 60° C. and heated at this temperature for one hour. Again shake. The constricted sealed end is again filed off and a few drops shaken out in a watch glass for standardization, and at the same time a few drops are deposited on an agar slant as a test for sterility. (Incubation for twenty-four hours should not show growth.)

Wright found that by taking a definite quantity of blood and a similar quantity of bacterial emulsion, mixing the blood and bacterial emulsion, then making a smear and staining, it was possible to determine the ratio of bacteria to red cells, and from this the number of bacteria per cubic centimeter could be determined. For example, if we find three bacteria to each red cell we should have 15,000,000 bacteria to 1 cubic millimeter. (There being 5,000,000 red cells to the cubic millimeter.) As 1 cubic centimeter is 1000 times greater than 1 cubic millimeter, there would be 15,000,000,000 bacteria in each cubic centimeter of such an emulsion, or vaccine, as it is termed. The standardization may be made with a hæmacytometer.*

Having determined the strength of the stock vaccine, we should prepare a dilute vaccine for injection. This is most conveniently carried out by filling vials with 50 c.c. of salt solution, plugging with cotton, then sterilizing in the autoclave. A sterile rubber cap is now drawn over the mouth of the vial. Sterility is insured by plunging the rubber cap and neck in boiling water. If the stock vaccine showed 5,000,000,000 bacteria per c.c. and we desired to have a vaccine containing 2,000,000,000 bacteria per c.c., it would only be necessary to draw out 2 c.c. of the salt solution by means of a sterile syringe needle inserted through the rubber cap and replace it with 2 c.c. of the bacterial emulsion. Example: In introducing 2 c.c. of a vaccine containing 5,000,000,000 bacteria per c.c., we throw in 10,000,000,000 bacteria in a volume equal to 50 c.c. Then each c.c. of the 50 c.c. in the bottle would contain 10,000,000,000 divided by 50 or 200,000,000 in each c.c. If we only want a vaccine containing 100,000,000 per c.c. we should only add 1 c.c. We now add 1/4% of trikresol to the vaccine in order to insure sterility. (Introduced with syringe, inserting needle through rubber cap.) The syringe is best sterilized by drawing up vaseline

* This is best done by drawing up the vaccine to .5 with either the red or white pipette, according to concentration, and then sucking up 1 to 10 dilute carbol fuchsin to 11 or 101. Allow the bacteria to settle on the shelf for ten minutes before counting. *Count as in making* a red count.

or olive oil heated to 150° C., and the neck and rubber cap of the bottle in boiling water. We now draw up the desired dose of bacteria. If glass syringes are used, simply boiling in water suffices. The ordinary doses are: For gonococci, streptococci, pneumococci, and colon vaccines, 5,000,000 to 50,000,000. For staphylococci 200,000,000 to 1,000,000,000.

ANAPHYLAXIS.

This is a term which indicates the opposite of prophylaxis. Our attention to this phenomenon commenced with the study of "serum sickness." In this it was noted that after a period of incubation of at least ten days a second injection of horse serum produced symptoms of respiratory embarrassment, convulsions and, at times, death. The primary injection had during the period of incubation sensitized the cells to this particular proteid.

Immunization against anaphylaxis is possible by repeating injection of the sensitizing serum or proteid during the period of incubation.

Anaphylaxis or hypersusceptibility is transmitted through the female to the offspring.

It is important to note that this hypersusceptibility appears to be of very little practical importance in the matter of the administration of a second injection of diphtheria antitoxin after the period of anaphylactic incubation.

As a rule the death or untoward effects of the injection of serum are in cases of status lymphaticus.

The phenomenon is peculiarly prominent in experimental work on guinea-pigs.

The name "Anaphylactine" has been applied to the sensitizing substance produced during the period of incubation.

It has been proposed to employ this phenomenon as a diagnostic measure. By taking the serum of a tuberculous patient, which would contain the sensitizing substance, and injecting it into the peritoneal cavity of a rabbit, the animal would be sensitized and an injection of tuberculin a few hours later would bring about the phenomena of anaphylaxis in the rabbit.

NOTES ON BACTERIOLOGY.

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PART II.
STUDY OF THE BLOOD.

CHAPTER XIII.

MICROMETRY AND BLOOD PREPARATIONS.

MICROMETRY.

IN the examination of blood and fæces preparations, especially when the identification of animal parasites is in question, there is nothing that assists more than a knowledge of the measurements of the object studied. The making of such measurements microscopically is termed micrometry.

Micrometry is also indispensable in bacteriology and cytodiagnosis as well as in animal parasitology.

The most practical way of making these measurements is with an ocular micrometer. These can be bought separately, or a glass disc (disc micrometer) with lines ruled on it can be dropped into the ocular to rest on the diaphragm inside the ocular. The ruled surface of this glass diaphragm should be placed downward. As was stated in connection with the microscope, the image of the object is formed at the level of the diaphragm rim inside the ocular, consequently the lines of the image cut those of the lines ruled on the glass in the ocular. Once having standardized the value of the spaces of the ocular micrometer for each different objective, all that is necessary subsequently in measuring is to count the number of lines or spaces which the image of the object fills and then, knowing the value of each space for that objective, to multiply the number of spaces by the value of a single space.

The unit in micrometry is the micron. This is usually written μ and is the 1/1000 part of a millimeter. There are 1000 microns in a millimeter.

To standardize: For this purpose it is necessary to have a scale of known measurements. The stage micrometers are usually ruled

in spaces of .1 and .01 mm. The lines which are 1/10 of a millimeter apart are consequently separated by a distance of 100 microns; those 1/100 of a millimeter apart are separated by a distance of 10 microns.

The ocular micrometer is usually ruled with 50 or 100 lines or spaces, separated by longer lines into groups of 5 and 10.

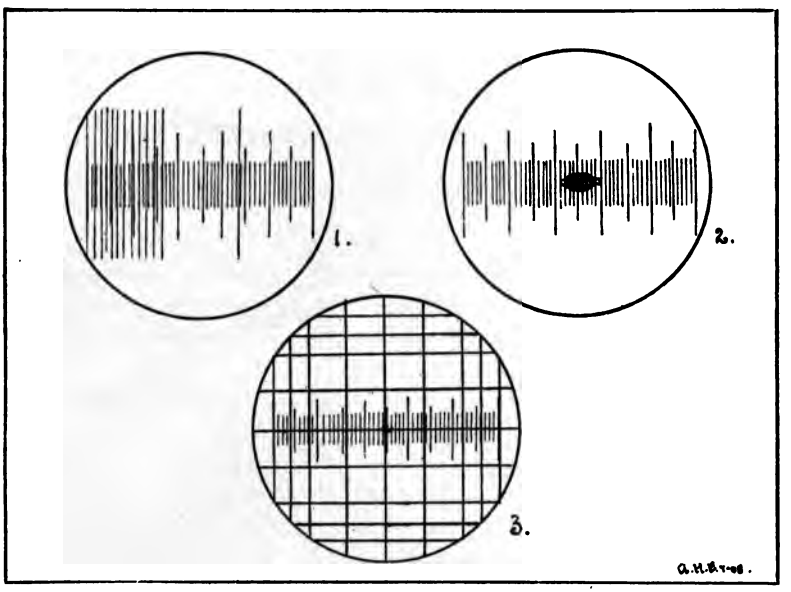


FIG. 48.—Micrometry diagrams. 1. Ocular micrometer with stage micrometer. 50 spaces of ocular micrometer cover two 100 micron spaces and ten 10 micron spaces; equal 300 microns. Each division on ocular micrometer equals 6 microns. 2. Ocular micrometer subtending image of whip worm egg. 9 spaces of ocular micrometer cover Whipworm egg. Each space equals 6 microns. Whipworm egg equals 54 microns. 3. Ocular micrometer with ruling of hæmacytometer. 50 spaces of ocular micrometer cover space equal to width of 6 small squares $50 \times 6 = 300$ microns. Each division of ocular micrometer equals 6 microns.

Having brought the lines on the stage micrometer to a focus, we determine the number of spaces on the stage micrometer which the 50 divisions of the ocular micrometer cover. To distinguish the ruling of the ocular from that of the stage micrometer, revolve the ocular with the fingers.

The tube length which is used at the time of standardizing must always be adhered to in subsequent measurements.

Example: With a 2/3-inch objective, the 50 rulings of the ocular micrometer fill in fifteen of the 1/10 millimeter rulings (100μ) and three of the 1/100 millimeter spaces (10μ). Consequently the 50 spaces of the ocular cover 1530 microns (15 x 100 = 1500; 3 $\times$ 10 = 30). Then if 50 spaces equal 1530 microns, one space would equal 30.6 microns. With the 1/6-inch objective the 50 ocular spaces would cover about three of the 1/10 millimeter (100μ) spaces of the stage micrometer. Then the 50 spaces would equal 300 microns and one space would equal 6 microns.

The ruling of the slide of a Thoma-Zeiss hæmocytometer will answer as well as a stage micrometer. The small squares are 1/20 of a millimeter square, consequently the distance between the lines bordering the small square is 1/20 millimeter or 50 microns.

Now, if with the 1/6-inch objective, the 50 lines on the ocular fill in the spaces of six small squares, the length of such a space would be 50 $\times$ 6 = 300 microns. This divided by 50 spaces would equal 6μ.

Should there be 100 spaces on the ocular micrometer instead of 50, it would only be necessary to divide the length in microns of the ruled surface of the stage micrometer covered by the 100 lines of the ocular micrometer by 100. The quotient would give the value in microns of each space of such an ocular micrometer.

The most accurate instrument for measuring is the filar micrometer. These are expensive. Measurements can also be made with the camera lucida, but it takes considerable time to make the adjustments necessary, so that it is not convenient. With an ocular micrometer one can make measurements of blood-cells, amœbæ, etc., in a few seconds—it only being necessary to slip in the ocular micrometer.

Rule for determining the magnifying power of microscopic lenses: Measure the diameter of the lens of the objective in inches—the approximate equivalent focal distance is about twice the diameter. Dividing 10 by the equivalent focal distance gives the magnifying power of the lens. This should be multiplied by the number of times the ocular magnifies. Example: The diameter of the lens of the objective was found to measure 1/2 inch, the focal distance would then be about 1 inch. Dividing 10 by 1 we have 10 as the magnifying power of the lens of the objective. If we were using a No. 4 ocular, the magnifying power would be approximately forty.

Blood Preparations.

To obtain blood, except for blood cultures, use either a platino-iridium hypodermic needle which can be sterilized in the flame, a small lancet, or a surgical needle with cutting edge. A steel pen with one nib broken off or the glass needle of Wright may also be used. To make a glass needle, pull straight apart a piece of capillary tubing in a very small flame. Tap the fine point to break off the very delicate extremity. Scarcely any pain attends the use of such a needle. In puncturing either the tip of the finger or lobule of the ear a quick piano-touch-like stroke should be used. The ear is preferable, as it is less sensitive and there is less danger of infection. Before puncturing, the skin should be cleaned with 70% alcohol and allowed to dry. It is advisable to sterilize the needle before using it.

The first drop of blood which exudes should be taken up on the paper of the Tallquist hæmoglobinometer, using subsequent ones for the blood pipettes and smears. If it is necessary to make a complete blood examination, it is rather difficult to draw up the blood in the pipettes, dilute it, and then get material for fresh blood preparations and films without undue squeezing, which is to be avoided. Of course, fresh punctures can be made. Ordinarily, complete blood examinations are not called for. It is only a white count or a differential count or an examination for malaria that is required.

Hæmoglobin Estimation.

The most accurate instrument for this purpose is the Miescher modification of the v. Fleischl hæmoglobinometer. The glass wedge for comparison with the diluted blood is similar in each instrument, but by the use of a diluting pipette accurate dilutions are possible in the Miescher. There are two cells provided—one 12 millimeters high, the other 15 millimeters; the idea of this being to enable one to make separate comparisons and to select the central parts of the glass-wedge scale, where comparison is more accurate than at the ends. As these cells contain columns of diluted blood proportionately as 5 to 4, we should have similar readings when we multiply the reading on the scale with the 15 mm. cell by 4/5.

The mixing pipette is graduated with the marks 1/2, 2/3 and 1/1—*the first giving* a dilution of 1 to 400 (when the diluent, a .1% soda

solution, is drawn up to the mark above the bulb) the second of 1 to 300 and the last of 1 to 200.

Artificial light is necessary. There is a table accompanying each instrument which shows the value for that particular instrument in milligram per liter of hæmoglobin for any reading obtained on the scale.

The apparatus is expensive, requires considerable time and care in the making of estimations, and is exclusively an instrument for a well-equipped laboratory.

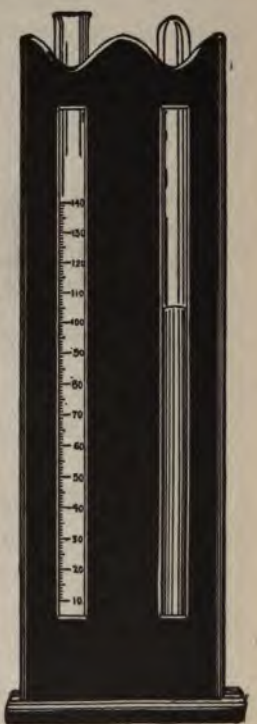


FIG. 49.—Sahli's hæmoglobinometer. (*Greene.*)

Sahli's Hæmometer.—A simple and apparently very scientific instrument which has been recently introduced is the Sahli modification of the Gower hæmoglobinometer. Instead of the tinted glass, or gelatin colored with picrocarmine to resemble a definite blood dilution, Sahli uses as a standard the same coloring matter as is present in the tube containing the blood. By acting on blood with ten times its volume of N/10 HCl, hæmatin hydrochlorate is produced, which gives a brownish-yellow color. In the standard tube, which is sealed, a dilution representing 1% of normal blood is used. To apply this test, pour in N/10 HCl to the mark 10 on the scale of the graduated tube. Add to this 20 cubic millimeters of the blood to be examined, drawn up by the capillary pipette provided. So soon as the mixture assumes a clear bright color, adds water drop by drop until the color of the tubes matches. The reading of the height of the aqueous dilution on the scale gives the Hb. reading. The tubes are encased in a vulcanite frame with rectangular apertures. This gives the same optical impression as would plano-parallel glass sides. It is recommended that the N/10 HCl be preserved with chloroform.

Tallquist's Hæmoglobin Scale.—This is a small book of specially prepared filter-paper with a color-scale plate of ten shades of blood colors. These are so tinted as to match blood taken up on a piece

of the filter-paper and are graded from 10 to 100. So soon as the blood on the filter-paper has lost its humid gloss, the comparison should be made. This is best done by shifting the blood-stained piece of filter-paper suddenly from one to the other of the holes cut in each shade—the piece of filter-paper being underneath the color plate. At least a square cm. of the filter paper should be stained by the blood. Daylight coming from a window to the rear or at the side should be used in making the comparison. The error with this method is probably not over 10% after a little experience. If the colored plate is not kept in the dark, the tints tend to fade.

To Count Blood-corpuscles.

The instrument almost universally used is the Thoma-Zeiss hæmacytometer. The apparatus consists of two pipettes, one for leukocytes, graduated to give a dilution of 1 to 10 or greater; the other for red cells to give a dilution of 1 to 100 or greater. The white pipette has the mark 11 above the bulb and the red pipette the mark 101. In addition, there is a counting chamber. This consists of a square of glass with a round hole in the center. Occupying the center of this round hole is a circular disc of glass of less diameter, so that an encircling channel is left. The square and the circle of glass are cemented to a heavy glass slide. The surfaces of each are absolutely level and highly polished. That of the circular disc is ruled into squares of varying size and is exactly 1/10 of a millimeter below the level of the surface of the surrounding glass square.

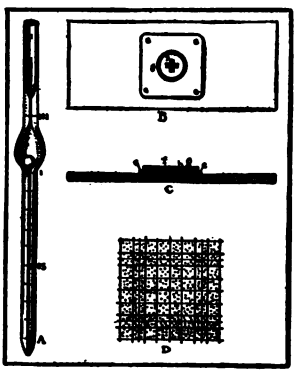


Fig. 50.—Thoma-Zeiss blood counter showing pipette, counting chamber, and ruled field. (*Greene.*)

When a polished plano-parallel cover-glass rests on the shelf, as the outer square glass is termed, there is a space left between its under surface and the ruled disk of .1 millimeter. The channel around the disk is termed the moat or ditch. The most desirable rulings are those of Turck and of Zappert. In these the entire ruled surface consists of nine large squares, each 1 millimeter square. These are subdivided, and in the central large square are to be found the small squares used for aver-

aging the red cells. These small squares are 1/20 of a millimeter square and are arranged in nine groups of sixteen small squares by bordering double-ruled lines. As the unit in blood counting is the cubic millimeter, if one counted all the white cells lying within one of the large squares (1 millimeter square), he would have only counted the cells in a layer 1/10 of the required depth, so that it would be necessary to multiply the number obtained by 10. This product, multiplied by the dilution of the blood, would give the number of white cells in a cubic millimeter of undiluted blood.

To make a red count: Having a fairly large drop of blood, apply the tip of the 101 pipette to it and, holding the pipette horizontally, carefully and slowly draw up with suction on the rubber tube a column of blood to exactly .5 or 1. The variation of 1/25 of an inch from the mark would make a difference of almost 3%. If the column goes above .5, it can be gently tapped down on a piece of filter-paper until the .5 line is cut. Now insert the tip of the pipette into some diluting fluid and, revolving the pipette on its long axis while filling it by suction, you continue until the mark 101 is reached. A variation of 1/25 of an inch at this mark would only give an error of about 1/30 of 1%. After mixing thoroughly by shaking for one or two minutes, the fluid in the pipette below the bulb is expelled (this, of course, is only diluting fluid). A drop of the diluted blood of a size just sufficient to cover the disc when the cover-glass is adjusted, is then deposited on the disc and the cover-glass applied by a sort of sliding movement, best obtained by using a forceps in one hand assisted by the thumb and index-finger of the other.

Among diluting fluids Toisson's is probably the best:

Sodium chloride,	1 gram
Sodium sulphate,	8 grams
Glycerine,	30 c.c.
Distilled water,	160 c.c.

Dissolve the sodium chloride and the sodium sulphate in the glycerine water and add sufficient methyl or gentian violet to give a rich violet tint.

A salt solution of about 2% strength, tinged with about 1 drop of a saturated alcoholic solution of gentian violet to about 50 c.c., is a good substitute, or the salt solution alone will answer when no white count is to be made at the same time as the red one.

It is important to work quickly in adjusting the cover-glass, or there

will be cells settling in the center of the drop from a greater depth than the one which the apposition of the cover-glass makes (1/10 millimeter deep).

A good preparation should show:

1. Presence of Newton's rings.
2. Absence of air bubbles.
3. Entire surface of ruled disk covered.
4. Equal distribution of cells.

Before counting, about five minutes should be allowed for the settling of the cells.

It will be remembered that the small squares are 1/20 millimeter square. The depth of fluid from upper surface of shelf to lower surface of cover-glass is 1/10 mm. Hence each space embraced by the small square and the depth of fluid is 1/4000 of the unit used in estimating number of corpuscles in blood, or 1 cubic millimeter ($1/20 \times 1/20 \times 1/10 = 1/4000$). Count 100 of the small squares (this enables one to use decimals). There are nine squares between double-ruled lines, each containing sixteen small squares. Count the number of corpuscles in the sixteen small squares contained in upper left-hand double-ruled square. Put down this count. Next count corpuscles in the adjoining sixteen squares. Put down this count. Then in third sixteen squares. Put down the number. Now move down to next row of three double-ruled squares. Count the number of corpuscles in each of the three sixteen-square spaces and set down the numbers for addition. We have now counted ninety-six small squares (6×16). Count at any place four additional small squares and add number of blood-cells contained therein to those in the ninety-six small squares already counted. Divide the sum by 100 or simply point off two decimals. This gives the average for each small square. Multiply this by the dilution and then (as the small square is only 1/4000 cu. mm.) by 4000. This will give the number of corpuscles in 1 cubic millimeter. Example: 100 small squares contained 655 red cells. Pointing off, 6.55 equals average number of red cells per small square. Multiply by dilution (200) and then by 4000 (the small square is 4000 times smaller than the unit: 1 cu. mm.)—$6.55 \times 200 = 1310 \times 4000 = 5{,}240{,}000$.

At least 100 small squares, and preferably 200 should be counted. If the blood appears normal, one may simply count the number of red *cells in five* of the sixteen small square spaces (eighty small squares). Having

added the numbers and multiplying by 10,000, you obtain the number of cells in 1 cubic millimeter. (Eighty small squares is 1/50 of the unit of 1 cu. mm., or 4000 small squares. The blood dilution being 1 to 200, we have 50 × 200 × number of cells in eighty small squares.)

In counting, count corpuscles lying on the lines above and to the right. Do not count those lying on lines below and to the left.

In the small squares count only corpuscles lying in the space or cutting the upper line. This prevents counting the same cell twice.

To Count White Cells.—Draw up the fluid in the white pipette to the mark .5. Then, still holding the pipette as near the horizontal as possible, because the column of blood tends to fall down in the larger bore, draw up by suction a diluting fluid which will disintegrate the red cells without injuring the whites. The best fluid is .3% of glacial acetic acid in water. This makes the white cells stand out as highly refractile bodies. Some prefer to tinge the fluid with gentian violet. The .5 mark is preferred because it takes a very large drop of blood to fill the tube up to the 1 mark and if there is much of a leukocytosis a 1 to 10 dilution is not sufficient. In leukæmic blood it is better to use the red pipette with the .3% acetic acid solution.

The blood having been drawn up to .5, we have a dilution of 1 to 20. Making a preparation, exactly as was done in the case of the red count, we count all of the white cells in one of the large squares (1 sq. mm.). The cross ruling greatly facilitates this. Note the number. Then count a second and a third large square. Strike an average for the large squares counted and multiply this by 10, as the depth of the fluid gives a content equal to only 1/10 of a cubic millimeter. Then multiply by the dilution. Example: First large square 50; second large square 70; third large square 60. Average 60. Then 60 × 10 × 20 = 12,000, the number of leukocytes in 1 cubic millimeter of blood. The count may be made with a low power (2/3-inch objective) as the leukocytes stand out like pearls. It is better, however, to use a higher power, so that pieces of foreign material may be recognized and not enumerated as white cells.

When it is desired to make a white count with the same preparation as is used for the red one, especially if the ruling is of the old style (only central ruling and not in nine large squares as with Zappert and Türck), it is advisable to make use of the method of counting by fields. With a Leitz No. 4 ocular and a No 6 objective, with a tube length of 120 milli-

meters, it will be observed that the field so obtained has a diameter of eight small squares. Now, remembering that he area of a circle equals the square of the radius multiplied by π, or 3.1416, we have the following calculation: The diameter being eight small squares, the radius would be four small squares. Squaring the radius, we have sixteen. This multiplied by 3.1416 gives us fifty. This means that every field, with the microscope adjusted as stated, contains fifty of the small squares, or 1/80 of the unit of one cubic millimeter of the diluted blood.

By keeping a single red cell in view while moving the mechanical stage from right to left or from above downward, we know that a new field of fifty small squares is brought into view when the circumference of the field cuts this individual cell. Example: As 2000 small squares would ordinarily be a sufficient number to count for a white count, this would require us to count the number of leukocytes in forty of the designated microscopic fields (this, of course, is only one-half the unit, hence we should multiply by 2). Counted forty fields and noted fifty white cells. $50 \times 2 = 100 \times 200$ (the dilution in red pipette) $= 20{,}000$. Consequently 20,000 would represent the number of leukocytes in one cubic millimeter of the blood examined.

After making a blood count, the hæmacytometer slide should be cleaned with soap and water and then rubbed dry, preferably with an old piece of linen. As the accuracy of the counting chamber depends upon the integrity of the cement, any reagent such as alcohol, xylol, etc., and, in particular, heat, will ruin the instrument. The pipettes should be cleaned by inserting the ends into the tube from a vacuum pump, as a Chapman pump. First draw water or 1% sod. carbonate solution through the pipette, then alcohol, then ether, and finally allow air to pass through to dry the interior. If the interior is stained, use 1% HCl in alcohol. If a vacuum pump is not at hand, a bicycle pump or suction by mouth will answer.

Preparations for the Study of Fresh Blood.

Many authorities prefer a fresh-blood specimen to a stained dried smear in the study of parasites of the blood. In malaria in particular there is so much information as to species to be obtained from a fresh specimen that the employment of this method should never be neglected. While waiting for the film to stain one has five or six minutes which

could not be better spent than in examining the fresh specimen which only requires a moment to make.

Manson's Method.—Have a perfectly clean cover-glass and slide. Touch the apex of the exuding drop of blood with the cover-glass and drop it on the center of the slide. The blood flows out in a film which exhibits an "empty zone" in the center. Surrounding this we have the "zone of scattered corpuscles," next the "single layer zone" and the "zone of rouleaux" at the periphery. It is well to ring the preparation with vaseline. When desiring to demonstrate the flagellated bodies in malaria, it is well to breathe on the cover-glass just prior to touching the drop of blood.

The Method of Ross is very easy of application and gives most satisfactory preparations. Take a perfectly clean slide, and make a vaseline ring or square of the size of the cover-glass. Then, having taken up the blood on the cover-glass, drop it so that its margin rests on the vaseline ring. Gently pressing down the cover-glass on the vaseline makes beautiful preparations which keep for a very long time. If it is desired to study the action of stains on living cells, this method is also applicable. A very practical way to do this is to tinge .85% salt solution containing 1% sodium citrate (the same as is used in opsonic work) with methylene azur, gentian violet, or methyl green. With a Wright bulb pipette, take up one part of blood, then one part of tinted salt solution. Mix them quickly on a slide and then deposit a small drop of the mixture in the center of the vaseline ring and immediately apply a cover-glass and press down the margins as before. This method will be found of great practical value.

A Method for Making Differential Leukocyte Count in same Preparation as for White Count.

Employ the same technic as in making the ordinary white count but using as a diluting fluid a 2% formalin solution to which has been added one drop of Giemsa's stain for each c.c. just before making the blood examination. The best results are obtained when the mixing in the pipette bulb is done immediately after taking up the blood and diluent.

The usual technic in making the hæmocytometer preparation is employed, using a Türck ruling. Count the leukocytes in the three upper or lower 1 sq. mm. squares, divide by 3 to obtain an average per

sq. m.m., multiply by 10 for the content of a cubic millimeter and then by 20 for the dilution. (Blood to .5; diluent to 11.) This can be done mentally and requires no calculation on paper. Having counted the leukocytes, again go over the same portion of the ruled surface and count the polymorphonuclears and estimate the percentage of these to the total leukocytes. The majority of disrupted cells in a dry-stained preparation are transitionals, hence the percentage of polymorphonuclears by this method is lower.

It is unnecessary in such a count to have an assistant; of course, in making a complete differential count it is preferable to have some one tabulate or laboriously to do this one's self.

The red cells are practically diaphanous and not disintegrated as when acetic acid is used as a diluent, consequently it is easy to make out the particular red cell as to size, etc., containing a malarial parasite.

The best results are obtained with a 1/6-in. objective. Higher powers are of course impracticable by reason of the thickness of the cover-glass of the hæmocytometer.

The following are the appearances of the various leukocytes.

Eosinophiles.—In these the bilobed nucleus stains rather faintly and the color is greenish blue. The eosinophile granules show easily as coarse, brickdust-colored particles.

Polymorphonuclears.—The nucleus stains a deep, rich, pure violet but less intense than that of the small lymphocyte. The shape of the nucleus is typically three or four lobed but even when of the horseshoe shape of a transitional nucleus is easily recognizable by the intensity of the violet staining. That which makes the polymorphonuclears very easy of differentiation is the distinctness of the cell outlines produced by the fine yellowish granulations in the cytoplasm.

Small Lymphocytes.—The nucleus is perfectly round and stains a deep violet. It is almost impossible to make out any cytoplasmic fringe.

Large Lymphocytes.—The nucleus here is round and of a lighter violet than that of the small lymphocyte. The cytoplasm is blue, non-granular, and sharply defined from the nucleus.

Large Mononuclears.—These show a washed-out, slate-colored nucleus which blends with the gray slate-blue staining of the cytoplasm so that there is an indefiniteness of outline in the more or less irregularly *contoured nucleus.*

Transitionals.—These show the same characteristics as the large mononuclears, but with a more faintly stained and more indented nucleus. The large mononuclears and transitionals stand out as slate-colored cell. When very much degenerated these cells have a greenish hue.

The young ring forms of malaria show as violet-blue areas in the red cells. When half-grown or approaching the merocyte stage, the containing red cell takes on a faint pink coloration, thereby differentiating it from the noninfected red cells. At the same time the parasite is extruded and has the appearance of a violet-blue body projecting from the margin of the red cell. It is as if a blue body were budding from a pink one.

It is an easy matter with this method to count the number of trypanosomes or malarial crescents in a cubic millimeter of blood.

Preparation and Staining of Dried Films.

When preparations are desired for a differential count, Ehrlich's method of making films is to be preferred, as the different types of leukocytes are more evenly distributed. In making smears by spreading, there is a tendency for the polymorphonuclears to be concentrated at the margin while lymphocytes remain in the central part of the film.

In Ehrlich's method we have perfectly clean dry cover-slips. Take up a small drop of blood without touching the surface of the ear or finger. Drop this cover-glass immediately on a second one and as soon as the blood runs out in a film, draw the two cover-slips apart in a plane parallel to the cover-glasses. Slide them apart. Ehrlich uses forceps to hold the cover-glasses to avoid moisture from the fingers.

Of the various methods of spreading films on slides there is none equal to that described by Daniels. In this the drop of blood is drawn along and not pushed along. The films are even, can be made of any desired thickness by changing the angle of the drawing slide, and there is little liability of crushing pathological cells. Take a small drop of blood on the end of a clean slide. Touch a second slide about 1/2 inch from end with the drop and as soon as the blood runs out along the line of the slide end, slide it at an angle of 45° to the other end of the horizontal slide. The blood is pulled or drawn behind the advancing edge of the advancing slide. An angle less than 45° makes a thinner film. One greater, a thicker film.

Some prefer to take up the drop of blood on the slide on which the

smear is to be made, about 1/2 inch from the end. Then apply the spreader slide and so soon as the drop runs along the end of the spreader slide proceed as above described.

Of the various methods of making smears by means of cigarette paper, rubber tissue, needles, etc., the best seems to be to take a piece of capillary glass tubing and use this instead of a needle in making the film. There is one advantage about the strip of cigarette paper touched

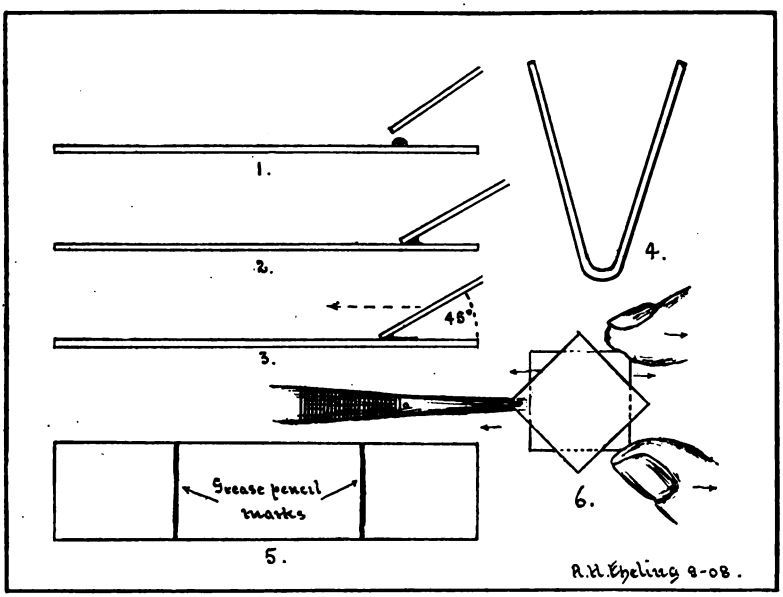


FIG. 51.—Blood technic. 1, 2, 3, Method for making blood smear on slide; 4, U tube for resting slides while staining; 5, slide showing grease pencil marking, marking prevents stain from overflowing; 6, method for drawing apart cover-glasses in making blood smear.

to the drop of blood and drawn out along the slide or cover-glass, and that is that it is almost impossible not to make a working preparation by this method.

In the making of smears the chief points are to make the smears as soon after taking the blood as possible and to have slides and cover-glasses scrupulously clean. It is well to flame all slides and cover-glasses which are to be used for blood-work. This is the best method of getting rid of grease.

Fixation of Film.—In Wright's, Leishman's, and other similar stains the methyl-alcohol solvent causes the fixation. In staining with Giemsa's stain, Ehrlich's tri-acid, hæmotoxylin and eosin, Smith's formol fuchsin, and with thionin, separate fixation is necessary. For Giemsa and thionin, either alcohol and ether (fifteen minutes), absolute alcohol (ten to fifteen minutes), or methyl alcohol (two to five minutes) answer well.

Formalin vapor, for five to ten seconds, is also used for fixation. For Ehrlich's tri-acid, hæmotoxylin and eosin and formol fuchsin, heat gives the best results. The best method is to place the films in an oven provided with a thermometer. Raise the temperature of the oven to 135° C. and then remove the burner. After the oven has cooled, take out the fixed slides or slips.

Some prefer to place a crystal of urea on the slide, then hold it over the flame until the urea melts. This shows that a temperature between 130° and 135° C. has been reached.

One of the handiest methods is to drop a few drops of 95% alcohol on the slide or cover-glass. Allow this to flow over the entire surface; then get rid of the excess of alcohol by touching the edge to a piece of filter-paper for a second or two. Then light the remaining alcohol film from the flame and allow the burning alcohol to burn itself out. A chemical fixation which gives good fixation for hæmatoxylin and tri-acid stains (not equal to heat) is a modification of Zenker's fluid (Whitney). To Muller's fluid, which is potassium bichromate 2 gms., sodium sulphate 1 gm., and water 100 c.c., add 5 gms. of bichloride of mercury and 5 c.c. of nitric acid (C. P.). Fixation is obtained in 5 seconds. Methyl alcohol for two minutes is satisfactory.

Staining Blood-films.—As separate staining with eosin and methylene blue rarely gives good preparations and as the modifications of the Romanowsky stain recommended are easy to make and employ, and give much greater information, the separate method of staining is not recommended. The most satisfactory single stain is thionin.

Rees' Thionin Solution.—Take of thionin 1.5 gms., alcohol 10 c.c., aqueous solution of carbolic acid (5%) 100 c.c. Keep this as a stock solution. It should be at least two weeks old before using. For use, filter off 5 c.c. and make up to 20 c.c. with water.

1. Fix films (a) by heat, (b) by alcohol and ether, or (c) preferably by 1% formalin in 95% alcohol for 1 minute.

2. Stain for from ten to twenty minutes. Wash and mount. Malarial parasites are stained purplish; nuclei of leukocytes, blue; red cells, faint greenish-blue.

Wright's Method.—The stain is made by adding 1 gram of methylene blue (Grubler) to 100 c.c. of a 1/2% solution of sodium bicarbonate in water. This mixture is heated for 1 hour in an Arnold sterilizer. When cool, add to the methylene-blue solution 500 c.c. of a 1 to 1000 eosin solution (yellow eosin, water soluble). Add the eosin solution slowly, stirring constantly until the blue color is lost and the mixture becomes purple with a yellow metallic luster on the surface, and there is formed a finely granular black precipitate. Collect this precipitate on a filter-paper and when thoroughly dry (dry in the incubator at 38° C.) dissolve .3 gm. in 100 c.c. of pure methyl alcohol (acetone free). This constitutes the stock solution. For use filter off 20 c.c. and add to the filtrate 5 c.c. of methyl alcohol.

A modification by Balch is very satisfactory. In this method instead of polychroming the methylene blue with sodium bicarbonate and heat, the method of Borrel is used. Dissolve 1 gm. of methylene blue in 100 c.c. of distilled water. Next dissolve .5 gram of silver nitrate in 50 c.c. of distilled water. To the silver solution add a 2 to 5% caustic soda solution until the silver oxide is completely precipitated. Wash the precipitated silver oxide several times with distilled water. This is best accomplished by pouring the wash-water on the heavy black precipitate in the flask, agitating, then decanting and again pouring on water. After removing all excess of alkali by repeated washings, add the methylene-blue solution to the precipitated silver oxide in the flask. Allow to stand about ten days, occasionally shaking until a purplish color develops. The process may be hastened in an incubator. When polychroming is complete, filter off and add to the filtrate the 1 to 1000 eosin solution and proceed exactly as with Wright's stain.

In Leishman's method the polychroming is accomplished by adding 1 gram of methylene-blue to 100 c.c. of a 1/2% solution of sodium carbonate. This is kept at 65° C. for twelve hours and allowed to stand at room temperature for ten days before the eosin solution is added. The succeeding steps are as for Wright's stain.

The modification of the Romanowsky stain which can be recommended as giving good results with the least expenditure of time in making is that

of Hospital Steward R. W. King, U. S. Navy. The preparation of the stain is as follows:

Dissolve 1 gram of Grubler's methylene blue and 0.5 gram of sodium bicarbonate in 100 c.c. of distilled water. Transfer to porcelain dish and evaporate to dryness over Bunsen burner or alcohol flame. The fluid may be allowed to boil very gently until about half of the water has escaped, when the heat should be somewhat reduced. Evaporation is now facilitated by causing the fluid to flow upon the sides of the dish by tilting. This also overcomes the tendency to spluttering. The heat should be continued until the last trace of moisture has disappeared.

(If larger quantities than the above are to be made, separate dishes must be used.)

When perfectly dry, scrape off the stain with a spatula and transfer it to a 200 c.c. flask, then wash out the porcelain dish with successive 25 c.c. portions of 95% alcohol so that we have finally 100 c.c. of alcohol in the flask. Shake the contents of the flask from time to time and after a few hours filter. Next evaporate this alcoholic solution of the polychrome blue (the filtrate) to dryness in a porcelain dish over a water bath or carefully over a small flame. This dry stain is practically neutral. For use dissolve 0.2 gram of this "blue by alcohol," as the polychrome blue is termed, and 0.16 gram yellow eosin in 100 c.c. C. P. methyl alcohol.

In all Romanowsky methods distilled water should be used. If not obtainable, the best substitute is rain-water collected in the open and not from a roof.

Method of staining:

1. Make films and air dry.

2. Cover dry film preparation with the methyl-alcohol stain for one minute (to fix).

3. Add water to the stain on the cover-glass or slide, drop by drop, until a yellow metallic scum begins to form. It is advisable to add the drops of water rapidly in order to eliminate precipitates on the stained film. Practically, we may add 1 drop of water for every drop of stain used.

4. Wash thoroughly in water until the film has a pinkish tint.

5. Dry with filter-paper and mount.

Red cells are stained orange to pink; nuclei, shades of violet; eosino-

phile granules, red; neutrophile granules, yellow to lilac; blood platelets, purplish; malarial parasites, blue; chromatin, metallic-red to rose-pink.

Giemsa's Modification of the Romanowsky Method.—This is one of the most perfect of the modifications. The objection is that greater time in staining films is required than with the Wright or Leishman method and the stain is very expensive.

Take of Azur II eosin 0.3 gram. Azur II 0.08 gram.

Dissolve this amount of dry powder in 25 c.c. of glycerine at 60° C. Then add 25 c.c. of methyl-alcohol at the same temperature. Allow the glycerine methyl-alcohol solution to stand overnight and then filter. This is the stock stain. To use: Dilute 1 c.c. with 10 to 15 c.c. of distilled water. If 1 to 1000 potassium carbonate solution is used instead of water it stains more deeply.

The alkaline diluent is used to obtain the coarse stippling in malignant tertian (Maurer's clefts). Having fixed the smear with methyl alcohol for five minutes, pour on the diluted stain, and after fifteen to thirty minutes wash off and continue washing with distilled water until the film has a slight pink tinge. For Treponema pallidum stain from two to twelve hours.

While the Romanowsky methods are more satisfactory for differential counts and for the demonstration of the malarial parasites, and especially for differentiating species, yet by reason of the liability to deterioration in the tropics of methylene blue the hæmatoxylin methods may be preferable. Many workers in blood-work and cytodiagnosis prefer the hæmatoxylin.

1. Fix the film either by heat with methyl alcohol for two minutes or with Whitney's fixative. Heat is to be preferred.
2. Stain with Meyer's hemalum or Delafield's hæmatoxylin for from five to fifteen minutes according to the stain. Frequently three minutes will be found sufficient. To make the hemalum, dissolve .5 gram of hæmatin in 25 c.c. of 95% alcohol. Next dissolve 25 grams of ammonia alum in 500 c.c. of distilled water. Mix the two solutions and allow to ripen for a few days. The stain should be satisfactory in two or three days.

   To make Delafield's hæmatoxylin, dissolve 1 gram of hæmatoxylin crystals in 6 c.c. of 95% alcohol. Add this to 100

c.c. of saturated aqueous solution of ammonia alum. After exposure to light for a week, the color changes to a deep blue-purple. Add to this ripened stain 25 c.c. of glycerine and 25 c.c. of methyl-alcohol and, after it has stood for about two days, filter. The stain should be filtered from time to time as a sediment forms. This makes a stock solution which should be diluted 10 to 15 times with water when staining.

Mink's Modification of Unna's Hæmatoxylin.

Hæmatoxylin,	1 gram.
Alum,	8 grams.
Sulphur (sublimed),	1 gram.
Glycerine,	30 c.c.
Alcohol,	50 c.c.
Water,	100 c.c.

Dissolve the hæmatoxylin in the glycerine in a mortar. Dissolve the alum in the water and add it to the glycerine hæmatoxylin in the mortar. Then add the sulphur and the alcohol. The solution ripens in about three to four days. Allow the sediment to remain in the bottom of the bottle containing the stain and filter off small quantities as needed.

3. Wash for two to five minutes in tap water to develop the hæmatoxylin color.
4. Stain either with a 1 to 1000 aqueous solution of eosin or with a 1/2 of 1% eosin solution in 70% alcohol. The eosin staining only requires fifteen to thirty seconds.
5. Wash and examine.

IODOPHILIA.

This reaction is supposed to be due to the presence of glycogen, especially in the polymorphonuclears, in suppurative conditions.

Make blood-smears on cover-glasses as usual, and after they dry, but without fixation, mount them in a drop of the following solution:

Iodine,	1 part.
Potassium iodide,	3 parts.
Gum arabic,	50 parts.
Water,	100 parts.

Small brown masses in the polymorphonuclears or lying extracellular indicate a positive iodophilia.

Viscosity of the Blood.—This is estimated by observing the relative height to which blood rises in capillary tubes as compared with water, and normally varies from three to five. The higher the hæmoglobin content the greater the viscosity. Viscosity is high in arterio-sclerosis and diabetic coma, low in the anæmias of nephritis.

Coagulation Rate of Blood.—This determination is of value in connection with operations on jaundiced patients.

Wright's coagulometer is a standard instrument but is cumbersome.

A simple method of determining the rate is to take a piece of capillary glass tubing and holding it downward from the puncture to let it fill for three or four inches. Then at intervals of thirty seconds scratch the capillary tubing at short distances and break off between the fingers. When coagulation has taken place a long worm-like coagulum is obtained. Normally coagulation occurs in about three to four minutes.

Occult Blood.

The spectroscopic examination is quick but in urine will only show blood in a dilution of about 1 to 300. Teichman's acid-hæmin-crystals test is not very satisfactory for clinical work. A practical method is the following:

Mix well 2 grams calcium phosphate with 5 c.c. urine in a centrifuge tube with a glass rod. Now fill the tube with alkaline alcohol (10 c.c. 10% aqueous solution NaOH in 90 c.c. of 95 per cent. alcohol) and mix again. Throw down the sediment in the centrifuge tube for one minute. Pour off the supernatant fluid and again add fresh alkaline alcohol; centrifuge, and again pour off the supernatant fluid. Now add 5 c.c. of acid alcohol (40 c.c. glacial acetic acid to 60 c.c. 95% alcohol) to the sediment, mix thoroughly, and centrifuge for a few seconds. This supernatant fluid contains the hæmoglobin as hæmatin. Pour this Hb. solution into a test-tube and add 10 drops of freshly prepared tincture of guaiac or a knife point full of freshly powdered guaiac. Next add hydrogen peroxide drop by drop until 10 to 15 drops are added. A blue or greenish-blue color will result if blood be present.

For the benzidin test add about 3 grains of benzidin and then drop in 15 to 30 drops of hydrogen peroxide. A blue or bluish-green color is positive.

Some prefer the aloin test. In this the acid alcohol supernatant

fluid is treated with 10 to 20 drops of a 3% solution of aloin in 70% alcohol after first adding to the acid alcohol about 20 drops of old oil of turpentine or an equal amount of solution hydrogen peroxide. If blood be present a bright red color changing to a cherry-red soon develops.

For fæces, if they are not liquid, rub up a small portion with sufficient water to make liquid. Treat as for urine. The same method answers for gastric contents. In examining the fæces for occult blood the patient should be on a meat-free diet for forty-eight hours. The diet period may be noted by giving a charcoal powder.

CHAPTER XIV.

NORMAL AND PATHOLOGICAL BLOOD.

In considering what may be termed normal blood, it must be borne in mind that the normal varies for men, women, and children:

	Hb.	Red cells.	Leukocytes.
Men,	90 to 110%,	5 to 5 1/2 million,	7500.
Women,	80 to 100%,	4 1/2 to 5 million,	7500.
Children,	70 to 80%,	4 1/2 to 5 million,	9000.

Color index.

This is obtained by dividing the percentage of the hæmoglobin by the percentage of red cells, five million red cells being considered as 100%. To obtain the percentage of red cells it is only necessary to multiply the two extreme figures to the left by two. Thus if a count showed the presence of 1,700,000 red cells, the percentage would be 34 ($17 \times 2 = 34$). If the Hb. percentage in this case were 50; then the color index would be $50 \div 34$, or 1.4.

In normal blood the color index is, approximately, 1.

In anæmias we have three types of color index: 1. The pernicious anæmia type, which is above 1. Here we have a greater reduction in red cells than we have of the hæmoglobin content of each cell. 2. The normal type, when both red cells and hæmoglobin are proportionally decreased, as in anæmia following hæmorrhage. 3. The chlorotic type. Here there is a great decrease in hæmoglobin percentage, but only a moderate decrease in the number of red cells. Hence the color index is only a fraction of 1. For example, in a case of chlorosis we have 40% of hæmoglobin and 90% of red cells, $40 \div 90 = .4$.

Red Cells.

In considering the corpuscular richness of a specimen of blood, it must be remembered that this does not necessarily bear any relation to *the quantity of* blood in the body. Thus, a more or less bloodless-

looking individual, the total quantity of whose blood is greatly reduced, may, notwithstanding, give a normal red count. In examining a specimen of peripheral blood we get a qualitative, not a quantitative result.

Normally, we have an increase in red cells in those living at high altitudes. An altitude of two thousand feet increases the red count about one million, and a height of six thousand feet about two million. Profuse sweats and diarrhœas also increase the red count. Pathologically, in chronic polycythemia with cyanosis and splenic enlargement, we have a red count of about ten million. In cyanosis from heart disease, etc., and in Addison's disease there is also an increase in red cells.

The normal red cell or erythrocyte measures about 7.5μ in diameter. It is nonnucleated and normally stains with acid dyes, taking the pink of eosin or the orange of orange G. If larger, 10 to 20μ, it is called a macrocyte; if smaller, 3 to 6μ, a microcyte.

Anisocytosis is a term applied to a condition where marked variation in size of the red cells occurs.

Macrocytes are rather indicative of severe forms of anæmia, the microcytes, of less grave types. When the red cell is distorted in shape, it is called a poikilocyte. Care must be exercised that distorted shapes are not due to faulty technic. Crenation and vacuolation of red cells are marked in poorly prepared specimens.

In addition to variation in size and shape, we also have pathological variation in staining affinities.

Polychromatophilia.—This shows itself by red cells taking a brownish to a dirty blue tint, as is frequently seen in immature red cells, especially nucleated ones.

Granular basophilic degeneration (also termed punctate basophilia and stippling) refers to the presence of blue dots in the pink background of stained red cells. It is found in many severe anæmias, as pernicious anæmia, the leukæmias, malarial cachexia, etc. It is very characteristic of lead poisoning.

The nucleated red cell, while normal for the marrow, is always pathological for the blood of the peripheral circulation. Normoblasts have the diameter of a normal red cell. The nucleus is round and stains intensely with basic dyes, often appearing almost black. Another characteristic is that it frequently appears as does the setting in a ring. Some give the term microblast to smaller nucleated forms. In normoblasts the red cell proper stains normally. The megaloblasts not only

have a greater diameter than the normoblast, but the nucleus is poor in chromatin, stains less intensely and is less distinctly outlined. Instead of being round, the nucleus is irregular and may be trefoil in shape. The cytoplasm surrounding the nucleus shows polychromatophilia. This contrasted with the pure blue of the lymphocytes should differentiate. Normoblasts are found in secondary anæmias, and especially in myelogenous leukæmia. Megaloblasts are peculiarly characteristic of pernicious anæmia. Enormous megaloblasts are sometimes termed gigantoblasts.

In aplastic anæmia (a severe type of pernicious anæmia), in contrast to ordinary pernicious anæmia, nucleated reds are very rarely found. There is also very little poikilocytosis, and the color index is about normal. It is a rare, rapidly fatal anæmia, particularly of young women.

It does not show remissions, runs a rapid course, and is attended with a marked increase of lymphocytes. The bone marrow of the femur is yellow and homogeneous.

The term leukanæmia has been employed to describe conditions which partake of the characteristics of pernicious anæmia and leukæmia.

White Cells.

Owing to the conflicting views as to origin, nature, and functions of the various leukocytes, their classification is in a state of confusion. As regards the appearance of the cells, this of course varies as the stain used, and it requires considerable experience for a single individual to be able to positively recognize the difference between a lymphocyte and a large mononuclear when one specimen is stained with a Romanowsky stain, another with Ehrlich's triacid, and a third w th hæmatoxylin and eosin. This, of course, is intensified when different persons adhere to the method of staining which they prefer and are at a loss to appreciate differences which are brought out by some other stain used by some other person. Even with the same stain used with different specimens of blood we find the staining characteristics of various leukocytes imperceptibly merging the one into the other, so that at times it is impossible for one, even with his own standard of differentiation, to be sure whether he is dealing with a lymphocyte or a large mononuclear. The difficulty is even greater when we deal with Türck's irritation forms and with *myelocytes.*

Without going into the various granule stainings so thoroughly brought out by Ehrlich, we shall immediately take up the question of a practical classification for use in making a differential count. As the Romanowsky method of staining (Wright, Leishman, or Giemsa) gives us information not yielded by either hæmatoxylin and eosin or the triacid, the points of differentiation to be referred to in that which follows is with blood so stained.

In considering the staining affinities of different parts of the leukocytes, it is convenient to divide such into basic ones, acid ones, and those which may be said to be on the border line between these—the so-called neutrophilic affinities.

With Wright's stain we have the eosinophile or oxyphile affinity of the granules of eosinophiles for acid dyes, in this case eosin. The nuclei and basophile granules have affinities in greater or less degree for basic stains (the blue and the violet shading resulting from methylene blue as modified by polychroming). With the granules in the cytoplasm of the polymorphonuclears and neutrophilic myelocytes, and to a less extent in the transitional, we have a staining which merges into a yellowish-red on the one extreme and into a lilac on the other. As a standard, neutrophilic granules should be a mean of these extremes.

Not only by reason of the authority of Ehrlich, but because such a division gives all variations, which can then be combined by one preferring a simpler classification, it would seem proper to divide the normal leukocytes into:

1. **Small Lymphocytes.**—These are small round cells about the size of a red corpuscle with a large centrally placed, deeply violet staining nucleus and a narrow zone of cytoplasm. This cytoplasm may not be more than a mere crescentic fringe. This is the type of lymphocytes which makes up the greater proportion of the leukocytes in chronic lymphatic leukæmia. At times these cells seem to be composed of nucleus alone.

2. **Large Lymphocytes.**—These are of the same type as small lymphocytes, but possessing more cytoplasm. The nucleus, while round and taking a fairly deep rich violet stain, does not stain so deeply as the nucleus of the small lymphocytes. The cytoplasm is a clear, translucent, pure blue. It may contain pinkish granules known as azur granules, but these are of rather large size and do not mar the glass-like appearance. They are from 9 to 15μ in diameter and are common in children. In the acute lymphatic leukæmias they at times predominate.

3. **Large Mononuclears.**—These are large round or oval cells with a nucleus which has lost the richness of violet staining of the lymphocyte nucleus. The nucleus is furthermore frequently irregular in outline or may show the commencing indentation of the transitional nucleus.

There is not that sharp distinction between nucleus and cytoplasm that exists in the lymphocytes. The cytoplasm of the large mononuclear gives the impression of opacity, as if it were frosted glass instead of clear glass. The neutrophile mottling which begins to appear causes a disappearance of the pure blue character of the cytoplasm of the lymphocyte. It is principally by the washed-out staining of the nucleus and the opaque lilac of the cytoplasm that we differentiate them from the lymphocytes. They greatly resemble Türck's irritation forms or plasma cells and may be confused with myelocytes.

4. **Transitionals.**—These appear as but a later stage in the decay of the large mononuclears; the nucleus is more indented, frequently horseshoe-shaped, and has a washed-out violet shade of less intensity than that of the large mononuclears. These are the cells so often disrupted in smears.

These four kinds of cells are frequently referred to as the lymphocyte series, and although many authorities consider that the small lymphocyte represents a more mature cell than the others of this class, yet it is thought by others that the age of the cell increases as we go from small lymphocytes to large lymphocytes, thence to the large mononuclear; and then in the transitional we have the decrepit stage which precedes dissolution. The old view that the transitional was the precursor of the polymorphonuclear has few advocates at the present time.

While it is convenient to consider these hyaline cells as representing different stages in development, yet from a standpoint of immunity this is untenable. The large mononuclears and transitionals are the cells in which we find certain animal cells and pigment phagocytized, as is the case in malaria. These cells are the macrophages of Metchnikoff and are probably derived from the bone marrow.

The lymphocytes take origin from the lymphoid tissue, and very probably the large lymphocyte is a younger, more immature cell than the small lymphocyte. Consequently, we probably have marrow lymphocytes and gland lymphocytes.

In addition to the series of leukocytes just considered we have present normally in the blood three types of granular cells distinguished according to the staining affinity of their granules. These are:

1. **Polymorphonuclear Leukocytes.**—This cell normally constitutes the greater proportion of the leukocytes. It is an amœboid, actively phagocytic cell, about 10 or 12μ in diameter, and is the microphage of Metchnikoff. Bacteria are actively phagocytized by this cell, and it is the cell concerned in determining the opsonic power of blood to various bacteria. It has fine lilac granules which are termed neutrophilic (epsilon granules). The single nucleus is rich in character and is lobose like the kernel of an English walnut; frequently it resembles the letter z. These cells are derived from the neutrophilic myelocytes of the bone marrow. It is in these cells that the glycogen, or iodophil, granules appear in certain suppurative conditions.

2. **Eosinophile Leukocytes.**—These are very striking cells with coarse granules staining brilliantly pink, the eosinophile, oxyphile, or acidophile granules (alpha granules of Ehrlich). The cells are a little larger than the polymorphonuclears. The normal eosinophile is to be distinguished from the eosinophilic myelocyte by its possessing two distinct lobes in the nucleus. The nucleus of the myelocyte is round. The eosinophile is the cell so frequently increased in infections by intestinal animal parasites.

3. **Mast Cells.**—These also have coarse granules, but they stain a deep violet blue. Hence they are basophile granules (gamma granules). In fresh blood these granules do not show up very well, thus they can be distinguished from the highly refractile granules of the eosinophile. The trilobed nucleus stains less intensely than the granules. As a rule, the mast cell is about the size of a polymorphonuclear.

In a differential count of normal blood we find about the following percentages.

Polymorphonuclears,	65 to 70%,	about 5000 per c. mm.
Small lymphocytes,	20 to 25%,	about 1500 per c. mm.
Large lymphocytes,	5 to 10%,	about 500 per c. mm.
Large mononuclears,	1 to 2%,	about 100 per c. mm.
Transitionals,	2 to 4%,	about 200 per c. mm.
Eosinophiles,	1 to 2%,	about 100 per c. mm.
Mast cells,	1/4 to 1/2%,	about 25 per c. mm.

The leukocytes which are found in the peripheral circulation only in pathological conditions are:

1. **Neutrophilic Myelocytes.**—The common type is a large cell with a large centrally placed, feebly-staining nucleus. This may be recognized by the difficulty of distinguishing the nucleus from the cytoplasm, there being no sharp line separating these parts of the cell. They imperceptibly merge into one another. They differ from a large mononuclear in that the cytoplasm is distinctly dotted with neutrophile granules and that we cannot make out a distinct line of separation of a slightly irregular or indented nucleus from the surrounding slightly neutrophilic cytoplasm. Cornil has described a very large myelocyte with eccentrically placed nucleus and neutrophilic granules.

Myelocytes are at times found with both basophilic and neutrophilic granules, and may rarely be seen to have all three kinds of granules on a single myelocyte, acidophile, basophile, and neutrophile.

2. **Eosinophilic Myelocytes.**—These can be distinguished from normal eosinophiles by their possessing a single round nucleus, not bilobed. These myelocytes may be as large as a normal eosinophile, but frequently are no larger than a red cell.

The neutrophile myelocyte is characteristic of spleno-myelogenous leukæmia, the eosinophile one of myelogenic leukæmia. The occurrence of an occasional myelocyte is frequently noted in conditions having a leukocytosis. In diphtheria their presence in numbers is of bad prognostic import. Myelocytes are of diagnostic importance in metastases of malignant tumors.

3. **The Irritation Cell of Turck, or Plasma Cell.**—This cell has a faintly staining, eccentrically placed nucleus, and a dark opaque blue, frequently vacuolated, cytoplasm. They are usually recorded as large mononuclears.

Blood Platelets.

These are normally present in blood in the number of about 350,000 per cubic millimeter. They disintegrate very quickly after the blood is withdrawn. Wright has demonstrated that they are pinched-off projections of giant cells of the bone marrow. They consist only of protoplasm, no nuclear material. They do not contain hæmoglobin. In conditions where giant cells are less abundant, as in pernicious anæmia, the blood platelets are less abundant. In myelogenous leukemia they

are very abundant. They vary in size from 2 to 5μ according as a larger or smaller pseudopod of a giant cell has been broken off. Stained with Wright's stain, they are more purplish than blue and show thread-like projections. They are often mistaken for the protozoal causes of various diseases. Especially are they confused with malarial parasites when lying on a red cell. The blood plate has no brick-red chromatic material; it is purplish rather than blue, and has no pigment grains. It is advisable to compare these isolated blood plates with the larger or smaller aggregations scattered about the smears. In this way their true character is apparent. In addition to blood platelets, which in fresh blood can only be observed when a fixative is used, we have other confusing bodies. The hemokonia of Muller are small, highly refractile bodies showing active oscillatory movement. They are supposed to be cast-off granules of eosinophiles or other leukocytes. Pinched-off fragments of red cells may also appear as possible protozoal bodies.

LEUKOPENIA.

This is a term used to designate a reduction in the normal number of leukocytes. A leukocyte count of 5000 would represent a slight leukopenia; one of 2000, a marked leukopenia. In the later stages of typhoid, and in acute miliary tuberculosis, we expect a moderate leukopenia. Chronic alcoholism and chronic arsenic poisoning cause a reduction in the number of the white cells. Pernicious anæmia shows a marked leukopenia, as is also the case with Banti's disease. Two tropical diseases, Kala-azar and dengue, show a marked leukopenia, the counts often being below 2500. During the apyrexial period of malaria we may have a white count of 5000.

It has recently been claimed that a leukopenia with a coincident marked reduction in the lymphocytes is characteristic of measles and that this occurs several days before the Koplik spots appear.

Kocher notes that in exophthalmic goitre the leukocyte count is considerably diminished and that the polymorphonuclears are not much more than one-half the usual percentage while the percentage of the lymphocytes is almost double the normal.

EOSINOPHILIA.

Where the eosinophiles are increased to 5%, we have a moderate eosinophilia. In some cases of infection with intestinal parasites,

especially hook-worms, but also from other parasites, as round and whip-worms, we may have an eosinophilia of 30 to 50%. In Guam, among the natives, it is difficult to find an eosinophile count under 15%.

The eosinophilia of trichinosis is best known, and a combination of this blood finding with fever and marked pains of muscles, would justify the excision of a piece of muscle for examination for encysted embryos. In true asthma eosinophilia is marked, and its absence is of value in indicating other causes for the condition. Certain skin diseases, especially pemphigus, also show eosinophilia.

The proportion of eosinophiles in the blood of children is greater than in that of adults.

Increase of both eosinophiles and mast cells is found in myelogenous leukæmia.

Leukocytosis.

It is to an increase in the polymorphonuclears that this term is usually applied, the term lymphocytosis or eosinophilia being employed where white cells of eosinophile or lymphocyte nature are increased. We have physiological leukocytosis in the latter weeks of pregnancy, also in the new-born, and in connection with digestion.

Pathological Leukocytosis.—Pneumonia. In this disease we have a leukocytosis of 20,000 to 30,000 or higher. The eosinophiles are almost absent. A normal leukocyte count in pneumonia makes a prognosis unfavorable.

Septic processes. The leukocyte count is of great value, especially when we obtain a leukocytosis with 80 to 90% of polymorphonuclears, as in appendicitis, cholecystitis, or other suppurative conditions.

According to Cabot, leukocytosis varies in infections as follows:

1. Severe infection—good resistance; early, marked and persistent leukocytosis.
2. Slight infection—slight resistance; leukocytosis present, but not marked.
3. In fulminating infections we may have no increase in whites, but a higher percentage of polymorphonuclears.
4. Slight infection and good resistance may not be productive of leukocytosis.

It is in connection with the question of operation in appendicitis or similar conditions that the matter of a leukocyte count is of prime importance. If there be a leukocytosis but with less than 75% of polymorphonuclears it indicates an infection of little virulence or a walled-off process with an exacerbation. It is difficult to form an opinion when the polymorphonuclears are under 80%. Leukocytosis with polymorphonuclear percentage of 85 to 90 indicates immediate operation; percentages over 90 point to peritonitis and if with such percentages of polymorphonuclears there is absence of leukocytosis the prognosis is grave.

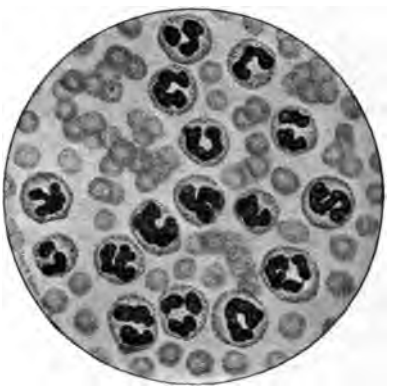


FIG. 52.—Leukocytosis (40,000); sixteen polymorphonuclears in field. (*Cabot.*)

Spirochæta fevers, as relapsing fever, may give a leukocytosis of from 25,000 to 50,000.

Small-pox, especially at time of pustulation, plague, scarlet fever, and liver abscess give a leukocytosis of from 12,000 to 15,000.

With meningitis counts of 25,000 are not unusual, in abscess of the brain the white count rarely exceeds 15,000.

Erysipelas and epidemic cerebrospinal meningitis also give a leukocytosis of from 15,000 to 20,000. In malignant diseases we sometimes have a moderate leukocytosis. Rogers states that in liver abscess, with a leukocytosis of 15,000 to 20,000, we have only about 75 to 77% of polymorphonuclears—there being also a moderate increase in the percentage of large mononuclears.

Lymphocytosis.

Of course, the disease in which we have the most marked lymphocytosis is lymphatic leukæmia.

Whooping-cough may give a lymphocytosis of 20,000 to 30,000.

Young children have normally an excessive proportion of lymphocytes. This is apt to be particularly marked in hereditary syphilis. Enlarged tonsils may give rise to a lymphocytosis of 10,000 to 15,000, when more than 50% of the white cells will be lymphocytes. Rickets and scurvy give a lymphocytosis.

Varicella and mumps may also give an increase in the percentage of lymphocytes.

Diseases in which there is a Normal Leukocyte Count.

Uncomplicated tuberculosis, influenza, Malta fever, measles, trypanosomiasis, malaria, syphilis, and chlorosis. In malaria we have a leukocytosis at the time of the rigor, while during the apyrexial period there is a moderate leukopenia. In malaria we have a marked increase in the percentage of the large mononuclears and transitionals. These may form from 25% to 35% of the leukocytes. When bearing particles of pigment they are known as melaniferous leukocytes—macrophages which have ingested malarial material. In dengue, at the time of the terminal rash, we may have as great a percentage of large mononuclears. In this disease, however, we have a great diminution of polymorphonuclears from the start (25 to 40%). Instead of a large mononuclear we have at the onset a lymphocytic increase. There is an increase of large mononuclears in trypanosomiasis.

The white count is about normal in uncinariasis (Ashford's average was 7800). Some have reported a leukopenia in severe cases.

While eosinophilia is the most marked feature in hook-worm disease yet in very severe cases it may be absent.

The Primary Anæmias.

Chlorosis.—In chlorosis it is the reduction of hæmoglobin with the slight numerical variation from normal of the red cells that makes for a diagnosis. The color index is very low. There is nothing abnormal about the leukocytes. Microcytes may be present, and very occasionally a normoblast. Macrocytes and megaloblasts are always absent.

Blood of chlorotics is very pale and very fluid and coagulates rapidly, hence frequency of thrombosis.

Spleen, liver, and lymph glands as a rule normal.

Simple Primary Anæmia.—This condition is not recognized by many authors, but is a convenient term under which to group anæmias which are neither chlorosis nor pernicious anæmia and for which no assignable cause can be designated. It is a secondary anæmia without a cause. In it color index is about normal, there is no change in the leukocytes and cases go on to recovery.

FIG. 53.—Pernicious anæmia. *M.m*, Megaoblasts; *n*, normoblast; *s*, stippling (punctate basophilia). (*Cabot.*)

Pernicious Anæmia.—In pernicious anæmia we obtain a very fluid, but normally colored drop of blood upon puncture. The yellow marrow of the long bones is transformed into a soft, bright red lymphoid tissue, smears from which show great numbers of megaloblasts. Areas of fatty degeneration are characteristic, especially the tiger-lily spots in the heart muscle. Iron-containing pigment (hemosiderin) is found in the liver, spleen, and kidneys. Areas of degeneration in the spinal cord may account for nervous symptoms. The red cells frequently fall below 2,000,000 with patients going about. Cases have been reported with counts under 200,000. The color index is high. Megaloblasts are the most characteristic qualitative change in the red cells. Megaloblastic crises may at certain times show enormous numbers of megaloblasts. Cases often present remissions in which no megalo-

blasts can be found. In such cases the presence of many macrocytes should prevent an examiner's reporting against a pernicious anæmia previously diagnosed.

Poikilocytosis, polychromatophilia, and stippling are also features of the disease. Normoblasts are far less frequent than megaloblasts and there is usually a moderate lymphocytosis. Myelocytes may be present. Cases of pernicious anæmia show remissions during which the patient is apparently on the road to recovery. Such improvements are only temporary. The remissions may last from two months to possibly three or four years. Especially in the anæmia of Dibothriocephalus latus do we have a picture of pernicious anæmia. It is supposed to be due to a toxin present in the heads of these tape-worms.

Secondary Anæmias.

These are the anæmias which can be definitely traced to some disease not of the hæmopoietic system.

There are two main groups—those following hæmorrhage and those secondary to various diseases. If the hæmorrhage is sudden and great, the resulting condition is one of oligochromæmia—chlorotic in type. Normoblasts are usually found after the third day. It is a question whether prolonged operation or those requiring narcosis are justified where the reduction in Hb. is under 40%. (According to Miculicz, 30% is the minimum).

Where the loss of blood is gradual; as in gastric cancer or severe hæmorrhoids, the picture may more nearly approach that of pernicious anæmia. Secondary anæmias usually show a moderate leukocytosis. In chronic nephritis and prolonged suppurative conditions normoblasts and macrocytes are rare—moderate poikilocytosis with the presence of many microcytes being the rule.

In fatal anæmias from chronic acetanilide poisoning high color index, macrocytes and megaloblasts have been noted.

In some secondary anæmias, as in syphilis, carcinoma, and tuberculosis, we have a chlorotic color index (chloro-anæmias).

In secondary anæmias polychromatophilia, poikilocytosis, and punctate basophilia (stippling) may be present. This latter is very marked in lead poisoning, but in certain cases of malarial cachexia it may be equally prominent. The only form of nucleated red cell seen is the normoblast, in very small numbers, or it may not be present.

Megaloblasts are practically never seen, except in some of the very severe parasitic anæmias, as the broad Russian tape-worm infection. The red cells generally number between 2,000,000 and 4,000,000, thus differentiating chlorosis. The leukocytes are frequently increased to 15,000. In the anæmia of splenic anæmia there is a marked leukopenia. In anæmias from malignant tumors the color-index is usually of the chlorotic type—the hæmoglobin content of the red cells being more affected than the number. Normoblasts are usually present, and this finding may differentiate gastric cancer from ulcer. In bone marrow metastases megaloblasts may be expected. Myelocytes and so-called tumor cells (large cells with faintly-staining vacuolated nuclei and but little cytoplasm) may also be found. As a rule, there is a moderate leukocytosis in malignant disease. Eosinophiles may be largely increased in sarcoma.

The Leukæmias.

It is in the leukæmias that we have the greatest increase in the number of white cells. These cases show more or less anæmia, but we may have cases of myelogenous leukæmia showing 250,000 leukocytes per cubic millimeter without particular change in the red cells. The more marked the red-cell change the more severe the condition.

There are two well-defined types of leukæmia, the lymphatic and the splenomyelogenous. It must be borne in mind, however, that while a greater change in the lymphatic glands may produce the lymphatic type, yet even in such cases we expect to find alteration in bone marrow and spleen; that is, there is a general involvement of the hæmopoietic system in all leukæmias, the activity being most marked in spleen and bone marrow in certain cases and in lymphatic glands in others.

Myelogenous leukæmia is a very rare disease, about five times as rare as pernicious anæmia. Lymphoid leukæmia is still more rare.

Splenomyelogenous Leukæmia (myeloid leukæmia).—The differentiation of the blood picture of this disease from leukocytosis does not depend on the number of leukocytes, but on the presence and large proportion of myelocytes. We expect both neutrophilic and eosinophilic myelocytes in myeloid leukæmia—the proportion of these varies, but, as a rule, the neutrophilic one is the common one. The blood in advanced cases is milky and shows a most marked buffy coat. The marrow is largely replaced by a yellow pyoid material. The spleen may weigh 10 pounds.

The leukocyte count is on the average from 200,000 to 500,000. Cases are reported of more than 1,000,000 white cells. The neutrophilic myelocytes make up about 30 to 40% of these and, about equal in number, are found the polymorphonuclears, while the percentage of the lymphocytes is decreased (2 to 5%) and normal eosinophiles, eosinophilic myelocytes, and large mononuclears make up the remaining percentages. We usually have great numbers of normoblasts. Megaloblasts may be rarely found. The red count is usually about 2,500,000 and the color index low.

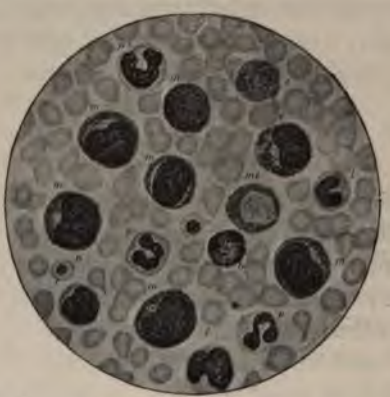


Fig. 54.—Myelogenous leukæmia. *m*, Myelocyte; *p*, polymorphonuclear; *b*, mast cell; *n*, normoblast. (*Cabot.*)

Lymphatic Leukæmia.—In this we have glandular enlargements, but not such large masses as in Hodgkin's disease. The red cells are usually reduced about one-half and the color index is a little below normal. Normoblasts are rarely found. Myelocytes, as a rule, are absent, but may amount to 5% of the leukocytes. The predominating leukocyte (75 to 98%) is the small lymphocyte. In acute lymphatic leukæmia the large lymphocytes predominate. The leukocyte count is never so great as in myeloid leukæmia, rarely exceeding 125,000.

Hodgkin's disease is usually considered as a disease with marked glandular enlargements, but with a negative blood picture. Undoubtedly the view that so-called lymphosarcomata, lymphatic leukæmia, and *Hodgkin's disease* merge into one another and that they represent a

malignant cell formation in the hæmopoietic system is the conservative one to take.

A certain proportion of cases of Hodgkin's disease, however, show endothelial proliferation and a chronic fibroid change.

Splenic anæmia is characterized by enlargement of the spleen and an unusually low color index.

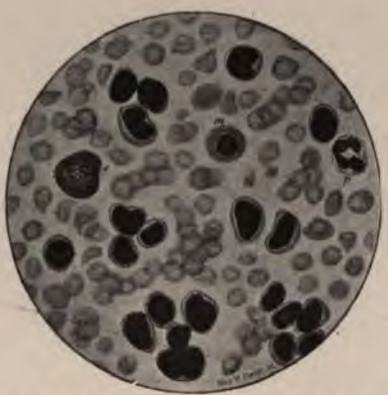


Fig. 55.—Lymphatic leukæmia. *p*, polymorphonuclear; *m*, megaloblast; *e*, eosinophile. Twenty-one lymphocytes in this field. (*Cabot.*)

There is a leukopenia.

Banti's disease also has a very low color index and leukopenia. In this the primary affection is of the spleen which becomes greatly enlarged. The accompanying cirrhosis of the liver with its symptoms of ascites, etc., differentiate it.

Anæmia infantum also gives a low color index and an intense anæmia. In this condition there is a lymphocytosis. The leukocytosis rarely exceeds 30,000.

NOTES ON BLOOD WORK.

Multiply average number cells in each small square by 800.000 provided dilution is 1-200 (blood drawn up to .5 mark and diluting fluid up to 101.) [illegible] (Ptl. p. 154).

WHITE CELLS.

Secure dilution of 1 to 10 by drawing the blood up to mark 1. Count all the leucocytes in all the squares (including those with double ruling)- of which there are 400.

Divide total number of cells by number of squares and multiply quotient by 4000 and this in turn by 10- the dilution. [illegible] large squares - take average in each large square + X 10 then multiply by [illegible] (Ptl. p. 155)

NOT GOOD NO SELLON

RED CELLS.

Multiply average number cells in each small square by 800.000 provided dilution is 1-200 (blood drawn up to .5 mark and diluting fluid up to 101.) [illegible] (Platt p. 154).

WHITE CELLS.

Secure dilution of 1 to 10 by drawing the blood up to mark 1. Count all the leucocytes in all the squares (including those with double ruling)- of which there are 400.

Divide total number of cells by number of squares and multiply quotient by 4000 and this in turn by 10- the dilution.

NOTES ON BLOOD WORK

NOTES ON BLOOD WORK.

PART III.
ANIMAL PARASITOLOGY.

CHAPTER XV.

GENERAL CONSIDERATIONS OF CLASSIFICATION AND METHODS.

ANIMALS that are in all respects alike we term a Species. Of course the male and female of a species may be very unlike, but as a result of mating they produce young having characteristics similar to the parents. Now, if, as in the case of the mosquitoes causing yellow fever, we find some with straight silvery lines and others uniformly showing crescentic silvery bands about thorax, yet resembling each other closely in the respect of being dark, brilliantly-marked mosquitoes, we should consider them as being separate species with a certain relationship to which the term Genus is applied.

The term "genus" is of wider application than the word "species." Thus animals which agree in the main characteristics of size, proportion of parts, and general structure are placed in the same genus.

In naming a species we always first write the name of the genus which has a Greek or Latin name, commencing with a capital, and follow with the specific term, which latter commences with a small letter. Thus we designate the dark silver-marked mosquitoes as belonging to the genus Stegomyia; those showing the characteristics of curved silver bands and two central parallel lines (lyre pattern) on dorsal surface of thorax we designate as Stegomyia calopus; the species with only the straight silver lines we call Stegomyia scutellaris.

Again, certain genera show resemblances which enable us to make broader groupings to which we apply the term Subfamily. Thus the genus Stegomyia and the genus Culex have the similar characteristics of palpi in the female being shorter than the straight proboscis; we therefore classify all species of Stegomyia and all species of Culex under the

designation Culicinæ. The name of a subfamily ends in "inæ." Now, again, certain insects are different from others in having scales on the wings. We find that not only do the Culicinæ have such characteristics, but the same is observed with the Anophelinæ and other similar scale-wing insects. All of these we term a Family and we speak of the Culicidæ, meaning the family of mosquitoes. The name of a family ends in "idæ." Many families are not subdivided into subfamilies, but are directly separated into genera. Again, a genus may have only a single species. When there are a number of families agreeing closely in some striking characteristic, we group them together into an Order; thus, the family of mosquitoes closely resembling many other families of insects in possessing a pair of well-developed wings are grouped in the order Diptera; all of which resemble certain other animals in the possession of a distinct head, thorax and abdomen with three pairs of legs projecting from the thorax. This collection of animals we call a Class; thus, we speak of the class Insecta. It will be observed that the insects have no internal skeleton, but instead a chitinous cuticle, the exoskeleton. Spiders, ticks, etc., resemble them in this respect, and we now apply to all such animals the wider designation, Branch or Phylum Arthropoda.

Inasmuch as the animal kingdom is divided into the branches Protozoa, Porifera, Cœlenterata, Echinodermata, Vermes, Arthropoda, Mollusca and Chordata, we see that the branch is the largest grouping we employ. To descend in the scale we have belonging to the branch, the classes; to the class, the orders; to the order, the families; to the family, the subfamilies; to the subfamily, the genera; to the genus, the species. Occasionally a species is further divided into subspecies.

By a type species we understand the species of a genus always referred to as representing the genus.

The male animal is designated by the sign of Mars (♂), the female by that of Venus (♀).

There are certain terms employed in animal parasitology which it is necessary to understand. Among these we shall refer to the following:

1. **True Parasitism.**—By this is understood the condition where the parasite does harm to the host, deriving all the benefit of the association. A good example of this would be the hookworm infecting man or animals.

2. **Mutualism.**—In such an association there is mutual benefit to *each party of the* association. An instance of this would be the presence

of colon bacilli in the intestines. The bacillus is furnished a suitable habitat and in return protects its host against strictly pathogenic bacteria.

Another example would be the oyster crab found inside the oyster shell.

3. **Commensalism.**—Here there is benefit to the parasite, but no injury to the host. An example of this kind would be furnished in the case of the Trichomonas vaginalis which lives in the vaginal mucus, but so far as known, does no injury to the host.

If the Entamœba coli be nonpathogenic this would be another example.

4. **Nomenclature.**—When the thousands of different species, genera, etc., of animals is considered, it will be readily perceived that, unless some system existed for their designation, indescribable confusion would prevail. To avoid this, the International Code, based on the rules of Linnæus (tenth edition of Systema naturæ, 1758, is basis of binary zoological nomenclature), requires Latin or Latinized names.

In printed matter the zoological name should be in italics, that of the family in Roman type. The name of the author of a specific name is written immediately after the name without punctuation and may be followed by the year of publication set off by a coma, thus: *Ascaris lumbricoides* Linnaeus, 1758. Should the name of the author appear in parentheses it indicates that he proposed the specific name but placed the species in another genus than that in which it now appears, and the name of the author responsible for placing the species in the present genus may be written after the name of the original author of the species; for example, *Davainea madagascariensis* (Davaine, 1869) Blanchard, 1891, tells us that Davaine proposed the specific name *madagascariensis* in 1869 but placed it in some other genus and that Blanchard in 1891 transferred it to the genus *Davainea*. There are certain rules governing the naming of animals. Of these, the law of priority provides that the oldest name, under the code, of any genus or species is its proper zoological name. The history of the naming of the organism of syphilis illustrates this well. Schaudinn gave this organism in 1905 the name of Spirochæta pallida. Ehrenburg, in 1838, had used the name Spirochæta for animals of a different character, so that this designation of the genus was not permissible under the code. Villemin, a little later, proposed the name of Spironema. This term, however, was found to have been used in 1864. Consequently it was not

available. Stiles then proposed the name Microspironema, but as Schaudinn only about two weeks before had offered the designation Treponema, the name Treponema pallidum had to be accepted as the proper zoological name for the organism of syphilis.

In case of larva and adult or male and female, formerly considered different animals but subsequently found to be the same, the oldest available name becomes the name of the species.

Another point is that names are not definitions, consequently the fact of lack of appropriateness of any name is no objection to its continuation. This will appeal to anyone as a wise provision, because if a different name were substituted each time a designation more descriptive or applicable was invented it would be utterly destructive to system. When it is considered that some of our parasites have approximately fifty different designations, for the most part given by medical observers, it will be appreciated how much the zoologist has aided us in trying to eliminate all but the single proper zoological name.

The objections so frequently heard among physicians in connection with adopting new names for old ones are not well founded. Wherever confusion has reigned, the establishment of order always results in temporary greater confusion. There is no doubt that the student taking up this subject a few years hence will have the satisfaction, thanks to the zoologist, of only having to burden his mind with one name for one parasite.

5. **Terminology.**—This applies to appropriate designations for different organs, symptoms, etc., and is not subject to any rule other than that of good usage.

Thus the terms cirrus in the case of the male copulatory organ of flukes, spicule for the same in nematodes and penis in connection with insects would be instances of terminology.

6. **Pseudoparasitism.**—Where organisms enter the body accidentally and when such sojourn in the body of man plays no part in the life history of the organism we employ the term pseudoparasitism. For example: Fly larvæ swallowed by man and passed out in the fæces. We also use the terms temporary parasites (bed-bug) and permanent parasites (liver fluke).

7. **Hosts.**—The animal in which a parasite undergoes its sexual life is called the definitive or final host, that in which it passes its larval existence the intermediary host. For example: Man is the intermediary

host of the malarial parasite, the mosquito the definitive host. A single animal may, however, be both definitive and intermediary host; thus Trichinella may pass its larval existence in the muscles of man and its sexual life in his intestines.

8. **Heredity, Congenitalism.**—Hereditary characteristics are those which were present in the ovum or spermatazoon before fertilization; congenital ones those which occur after fertilization. South African tick fever is probably an instance of heredity, the spirochætes having been found in the ovary and ova of the female tick.

9. **Heterogenesis.**—Offspring differs from parent, but after one or more generations there is reversion to the parent form.

CHAPTER XVI.

THE PROTOZOA.

Classification of Protozoa.

Class	Order	Genus	Species
Rhizopoda (Sarcodina) These throw out protoplasmic projections called pseudopodia.	Gymnamœba	Entamœba	E. coli E. histolytica E. buccalis
		Leydenia	L. gemmipara
Flagellata (Mastigophora) These move by means of undulating membranes or flagella.		Spirochæta	S. recurrentis S. vincenti S. duttoni S. carteri S. refringens
		Treponema	T. pallidum T. pertenue
		Trypanosoma	T. gambiense
		Trichomonas	T. vaginalis T. intestinalis
		Lamblia	L. intestinalis
		Babesia	B. bigemina
		Leishmania	L. donovani L. tropica
Infusoria (Ciliata) These have contractile vacuoles and numerous fine cilia which are shorter than flagella and have a sweeping stroke.	Heterotricha	Balantidium	B. coli
Sporozoa These have no motile organs. They live parasitically in the cells or tissues of other animals. Reproduction by spores.	Coccidiaria	Eimeria	E. stiedæ
		Isospora	I. bigemina
	Hæmosporidia	Plasmodium	P. vivax P. malariæ P. falciparum

THE PROTOZOA.

By the term protozoa we understand a branch of animals in which a single cell is morphologically and functionally complete; it is not one of a number of cells going to make up a complex individual and depend-

ent on such a combination as is the case with the metazoa (there is no differentiation into tissues in protozoa).

As a rule, protozoa have a nucleus contained within protoplasm which may generally be divided into a more hyaline outer portion (ectosarc) and a granular interior (endosarc). In addition to the chromatin in the nucleus we may have granules of chromatin outside the nucleus (chromidia).

Among the protozoa we may have new individuals produced from a female gamete without any fertilization by the male cell. Such a process is termed parthenogenesis. A very rare instance is where the male cell reproduces—termed etheogenesis.

RHIZOPODA (SARCODINA).

In this class of protozoa the pseudopodia serve the double purpose of nutrition and locomotion. These protoplasmic extensions may be quite broad or very narrow—the lobose and the reticulose.

As a rule, the thicker the pseudopod the more rapid the movement. Some rhizopods have hard shell-like coverings which are secreted in or on the ectosarc. These skeletons have openings through which the pseudopods project. The pseudopodia may be made up only of ectoplasm or both ectoplasm and endoplasm may take part. Amœboid movement always starts in the ectoplasm. In addition to the nucleus, which the so-called chromatin staining methods bring out as reddish areas, we frequently observe smaller aggregations of chromatin-staining material in the cytoplasm. This extranuclear chromatin is supposed to play a part in the more intricate divisions which such protozoa undergo. Food vacuoles and contractile vacuoles are present in many rhizopods.

Entamœba coli.—This is considered by Schaudinn to be a harmless inhabitant of the intestines and its presence in the fæces is not considered of importance. Musgrave and Clegg do not recognize a distinction between a nonpathogenic and a pathogenic amœba, but consider that the presence of amœbæ, in the absence of symptoms, is to be explained by the nonestablishment of a satisfactory symbiosis with some bacterium or other parasite. They state, that as a result of extended observation, persons harboring amœbæ will sooner or later develop dysentery. As regards morphological points of distinction, they state that even in pure symbiotic cultures, descended from a single amœba, the same variations

in size, motility, etc., may be observed. They also consider that amœbæ having all the characteristics of the harmless commensal may cause marked pathological change. Craig claims that the E. coli cannot be cultivated and, that several years since noting E. coli in stools of healthy persons, these persons have remained free of any dysenteric symptoms.

The only safe way in recognizing amœbæ in stools is to note amœboid

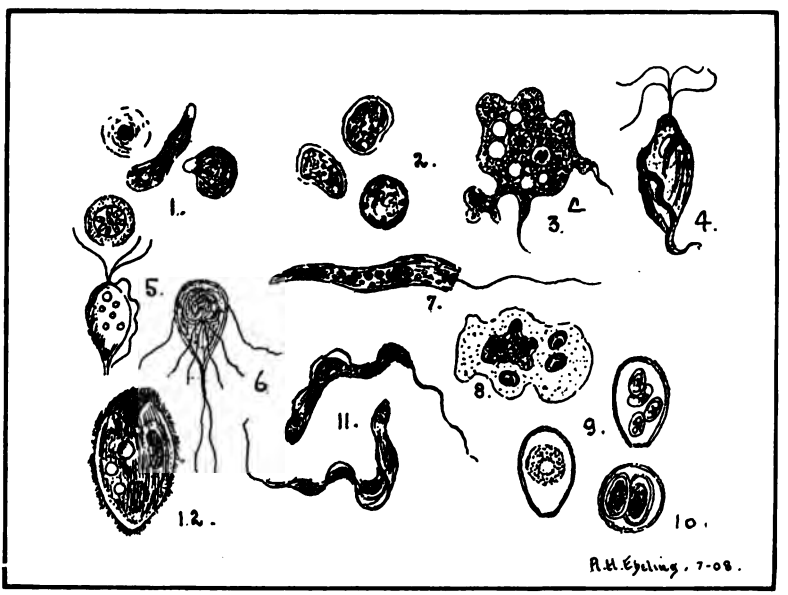


FIG. 56.—Various protozoa. 1, Entamœba coli; 2, Entamœba histolytica; 3, Leydenia gemmipara; 4, Trichomonas vaginalis; 5, Trichomonas intestinalis; 6, Lamblia intestinalis; 7, flagellated Leishmania donovani; 8, Leishmania donovani in phagocyte; 9, Eimeria stiedæ, 10, Isospora bigemina; 11, Trypanosoma gambiense; 12, Balantidium coli.

movement. The encysted amœbæ can scarcely be differentiated from many vegetable cells and especially from large phagocytic cells, of probable endothelial origin. By the use of neutral red in very dilute solution the granular endoplasm will be observed to take up the brick-red stain.

E. coli varies greatly in size (8 to 40μ). There is no well-marked distinction between a granular interior and a more compact, hyaline exterior. The nucleus is centrally situated, is distinct, and on staining *with Wright's* stain shows the chromatin coloration. It is sluggishly

motile and is of a grayish-white color. When stained it does not show a distinction between endoplasm and ectosarc. The infecting stage is an encysted form with eight amœbulæ.

Entamœba histolytica.—This is considered the pathogenic amœba. Schaudinn considers that it is by the possession of its tough, tenacious, glassy, and highly refractile ectoplasm that it is able to bore its way into the submucosa of the large intestine and bring about those gelatinous-like necroses, which, by undermining, eventually result in dysenteric ulcerations.

It is also the species found in tropical liver abscess. As described by Schaudinn, it has a marked differentiation between the glassy ectoplasm and the granular endoplasm. The nucleus is indistinct, eccentric, or even peripherally situated, and stains feebly.

The movement is more active and the color more greenish-yellow than E. coli. Craig notes the characteristic staining of the E. histolytica, this being a dark blue ectoplasm encircling a lighter blue endoplasm. In dividing, there is a process of budding. These little spore-like bodies form at the periphery of the encysted amœba and are the infecting stage. Cold or other inimical influences tend to make amœbæ encyst, hence fæces should be examined as soon as possible after the stool is passed. A particle of mucus pressed down with a cover-glass makes a satisfactory preparation. If necessary to dilute, use blood-warm salt solution—not plain water.

Entamœba buccalis.—This has an ectoplasm similar to E. histolytica, but has a centrally situated nucleus, the nucleus, however, is poor in chromatin.

Obtained from the mouths of persons with dental caries. It does not appear to have pathogenic characteristics.

Castellani has reported an intestinal amœba with an undulatory membrane. He has given it the name of E. undulans.

Leydenia gemmipara.—It is a question whether these bodies were animal parasites or simply body cells showing amœboid movement. They were found in the ascitic fluid of two cases of carcinomatosis. They varied in size from 3 to 36μ.

Flagellata (Mastigophora).

In this class of protozoa the adults have flagella for the purposes of locomotion and the obtaining of food.

Some flagellates more or less resemble rhizopods in being amœboid and in having an ectoplasm and an endoplasm. The body is frequently covered by a cuticle (periplast). Some flagellates have a definite mouth part, the cytostome, which leads to a blind œsophagus; others absorb food directly through the body wall. In addition to flagella, some flagellates possess an undulating membrane. All flagellates possess a nucleus and some have contractile vacuoles. The flagellum may arise directly from the nucleus or from a small kinetic nucleus, the blepharoplast (micronucleus or basal granule).

The most important flagellates of man are the hæmoflagellates. Among these we may include the blood spirochætes and the organism of syphilis, which have many resemblances to the spiral form of bacteria, together with the three genera in which protozoal characteristics are marked, namely, Leishmania, Trypanosoma and Trypanoplasma. In addition we have flagellates in the intestinal canal and in the vaginal secretion. Some authors place the genus Piroplasma with the flagellates and there has been controversy concerning the nature of certain projections from these bodies. It would seem preferable, however, to consider them under the sporozoa.

Spirochæta.

The generic term Spirochæta is applied to flagellates having a spiral shape, an undulating membrane, and no flagella. This genus is one about which there are two views: one, that the members belong to the bacteria; the other, that they are protozoa. The absence of demonstrable nucleus and blepharoplast make them apparently vegetable in nature while the variations in thickness, the fact of transmission by an arthropod, and indications of a longitudinal, rather than a transverse division, would indicate protozoal affinities.

It would seem from recent investigations that both methods occur—longitudinal division occurring when there are few organisms in the blood and transverse at the height of the infection.

S. recurrentis.—This is the organism of relapsing fever. It was formerly considered a bacterium and was termed the Spirochæta obermeieri (discovered by Obermeier in 1873).

It is present in the blood of persons suffering from the disease during *the pyrexia.* During the apyrexia they are not found in the peripheral

circulation. At this time they are present in great numbers in the spleen where they are actively phagocytized. The disease is supposed to be transmitted by bed-bugs or lice. Monkeys are susceptible and, after passage of the organism through monkeys, rats can be infected.

S. duttoni.—This is the cause of South African tick fever or "tete-fever." The disease is similar to relapsing fever, but there are generally four or five febrile paroxysms with apyrexial intervals. The disease is readily transmitted to ordinary laboratory animals, especially the rat.

A certain degree of immunity is conferred by an injection with a certain spirochæte, but this does not hold for other species; thus, rats

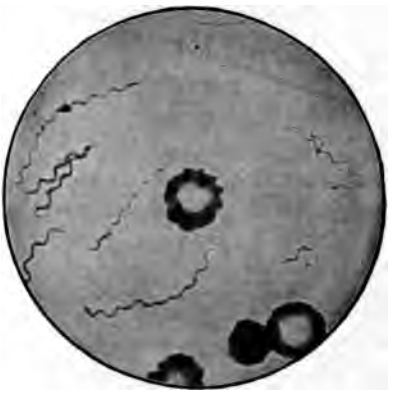


FIG. 57.—Spirochætæ of relapsing fever from blood of a man. (*Kolle and Wassermann.*)

which have recovered from S. recurrentis can be infected by S. duttoni and *vice versa*. The disease is transmitted by the bite either of the adult or larval Ornithodoros moubata. Koch found spirochætes in the eggs of the ovaries of ticks which had fed on persons with the disease. It is thus an instance of hereditary transmission.

Leishman, who believes in the protozoal nature of these organisms, has observed clumps of chromatin granules in the Malpighian tubes and in the ovaries of infected ticks, which granules he considers developmental stages. Material showing such granules but no spirochætes has brought about spirochæte infection in mice. He considers that infection probably occurs through material voided from the Malpighian tubes rather than through the medium of veneno-salivary secretions.

Other spirochætes that have been considered as pathogenic for the type of relapsing fever in India and that of America are the S. carteri and the S. novyi.

S. vincenti.—This is a very delicate spiral-shaped organism which has been found in conjunction with a fusiform bacillus in a throat inflammation, usually termed Vincent's angina.

S. refringens.—This Spirochæta is frequently associated with the Treponema pallidum and is common in genital ulcerations. It is thicker, has less regular and more flattened curves and stains more readily. By "dark ground illumination" it is thicker, of a yellow tint instead of pure white, and moves in its entire length.

Treponema.

The genus Treponema has no undulating membrane and has a flagellum at each end.

Treponema pallidum.—This is the cause of syphilis. It is characterized by the very geometric regularity of the spirals, which are deeply cut, and in focusing up and down continue in focus (like a corkscrew). They require about thirty minutes to stain distinctly with Giemsa's stain and the attenuated ends or flagella should always be noted before reporting their presence.

Treponemata are found in the cellular areas surrounding the thickened blood-vessels and in the coats of the larger arteries. To stain them in section Levaditi's method is the best.

The India-ink method of Burri is highly recommended. Take one loopful of secretion from a chancre and deposit it on one end of a slide. Surround this drop with five loopfuls of distilled water and five loopfuls of Günther and Wagner's ink. Mix and make a smear as for blood. When dry examine with the oil immersion objective and the treponemata will be found to stand out as white spirals against a dark background. Treponemata often appear as if bent in the middle.

T. pallidum has been cultivated anaerobically in horse serum by Schereschewsky. The cultures contained other organisms. Muhlens, by growing anaerobically on horse-serum agar (1 to 3), claims to have obtained pure cultures. Animal inoculations with this material were negative, however.

In diagnosis either use the dark ground illuminator or make a thin

smear from the sanious oozing after vigorous friction of the chancre with gauze, taking up this blood-stained serum on the end of a slide and smearing the surface of a second slide with the adhering material. It is in most cases more satisfactory to curet the lesion, in this way obtaining material from the areas of the thickened arteries. The smear is prefer-

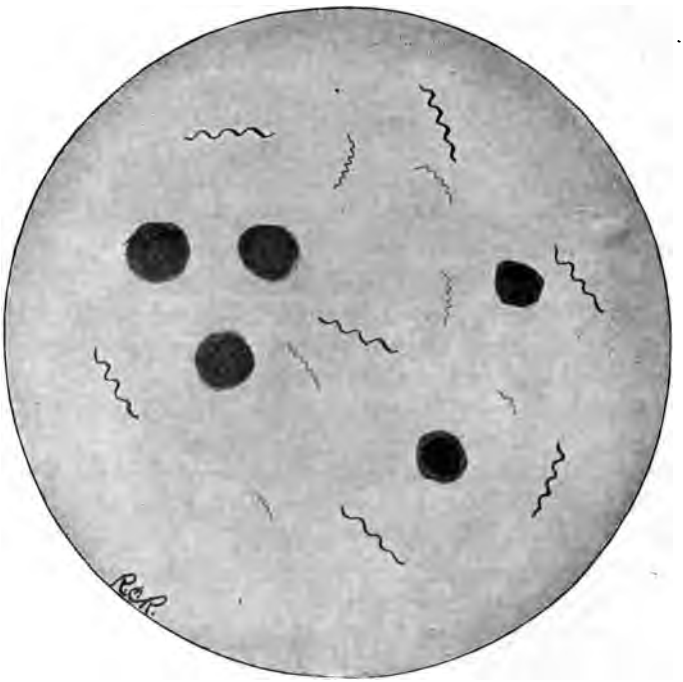


FIG. 58.—The Spirochæta refringens is the larger and more darkly stained organism, while the lightly stained and more delicate parasites are the Spirochæta pallida. (*Treponema pallidum*). From a chancre stained with Wright's blood stain. (*Hirsch—by Rosenberger.*)

ably fixed with heat. To 10 c.c. of a 5% solution of glycerine in a test-tube add thirteen drops of Giemsa's stain. Heat the contents to 60° C. by placing the test-tube in a beaker of water at 60° C.; pour this hot stain on the preparation and allow it to remain on for fifteen or thirty minutes. Wash in water. Great care should be taken to prevent any greasy material getting on the slide.

T. pertenue.—An organism of similar morphology was first reported

by Castellani as present in yaws. It is found in smears and sections as with T. pallidum.

A point of distinction between these spirochætes is that the T. pallidum is found in abundance in sections from a chancre about the thickened arteries in the corium, while in sections from a yaws nodule the T. pertenue is found chiefly in the region of the interpapillary pegs of the Malpighian layer of the epidermis where they bound the papillary layer of the corium.

Trypanosoma.

The genus Trypanosoma has a more or less spindle-shaped body, along one border of which runs an undulating membrane. There is one flagellum bordering the membrane and projecting like a whip posteriorly. There is a nucleus (macronucleus) and a blepharoplast (micronucleus—centrosome), the latter being located anteriorly as a chromatin staining dot or rod. From this blepharoplast the flagellum proceeds posteriorly bordering the undulating membrane and projecting freely beyond the posterior end. The nucleus is larger, nearer the posterior end, and does not stain so intensely as the blepharoplast.

Some consider that the trypanosome developed from types with a single anterior flagellum proceeding from a blepharoplast. The moving of the blepharoplast with the flagellum to the other end would make the flagellar end the anterior end. This controversy as to which is the anterior end is the cause of confusion.

T. gambiense.—This is the trypanosome causing human trypanosomiasis, the latter stage of which is known as sleeping sickness. It is from 17 to 28μ long, and from 1.5 to 2μ wide. Blepharoplast oval.

It was first discovered in smears from blood by Ford in 1901, and recognized as a trypanosome by Dutton in 1902, and observed in 1903 by Castellani in the cerebrospinal fluid of patients with sleeping sickness. It is now proposed to consider cases where trypanosomes are not present in the cerebrospinal fluid as in the first stage; when present, as in the second stage.

It is very difficult to distinguish the human trypanosome from some of the other pathogenic ones by staining methods. The immunity test is the most reliable. An animal recovered from an infection by a *certain* trypanosome does not possess immunity for other pathogenic

ones. Novy and McNeal cultivated T. lewisi in water of condensation on blood agar at room temperature, but up to the present T. gambiense has not been cultivated. It is present in the blood, usually in exceedingly small numbers, and in the lymphatic glands of patients. It is by puncture of the glands that we have the best means of finding the parasites. It is also found in the cerebrospinal fluid in sleeping sickness. The parasite stains readily with Wright's stain. The transmitting agent is the Glossina palpalis. It is not known whether this occurs by direct or indirect transmission. At any rate, there must be some peculiarity about the tsetse fly, either in the reaction of its salivary secretion or otherwise, to make it the only well-recognized agent in the spread of the disease. No tsetse fly, no trypanosomiasis. Koch observed certain developmental forms in the bulb of the proboscis, but whether they represented a developmental cycle or not is not settled.

Against this is the fact that a tsetse fly which has taken blood from an infected animal can only transmit the infection during the next twenty-four hours and that only to the first animal bitten—the infectious material apparently being disposed of in this single act of biting. Later work by Kleine, however, has shown that after a period of eighteen to twenty days the fly does become capable of transmitting the disease, and Bruce has shown that fluid from the gut of a fly fed seventy-five days previously on infected animals produced the disease in a monkey. Kleine has also shown that 5% to 10% of flies become infected after feeding on infected animals.

Other authorities think it possible that trypanosomes may encyst in the digestive tract, and so the flies transmit the disease along with their fæces. This does not seem to be possible in connection with human infections. Koch found several cases where infection had taken place by coitus. This is the method of infection in T. equiperdum, a trypanosome disease of horses.

The various trypanoscidal remedies, atoxyl, arsacetine, etc., have not proven very satisfactory. One of Ehrlich's latest products, arsenophenyl-glycine, however, has given encouraging results; horses affected with surra having been cured by its use. In man it has been given in doses of one gram without ill effects.

Trypanosoma cruzi Chagas, 1909.—A human trypanosomiasis found in the state of Minas, in Brazil, is caused by this protozoan. Cruz states that the specific protozoan is transmitted by a bug of the genus

Conorhinus (Reduviidæ). This trypanosome is capable of transmission to domestic animals as well as to man. It is remarkable for the large size of its kinetonucleus. The life history resembles that of T. gambiense, as nonflagellate, round forms composed of eight small trypanosomes can be found in the bugs. Each of these has a bilobed nucleus and, when the mass breaks up, each penetrates a red blood cell where it grows and from which it escapes as a full grown trypansome. Three forms are seen in the blood.

The symptomatology of this form of trypanosomiasis is that of anæmia (which has caused it to be confounded with ankylostomiasis) with wasted, stunted development, intermittent or remittent fever, œdemas of various areas, enlargement of lymph nodes (especially of neck and axilla) and splenic enlargement. Trypansomes are found in the blood during the febrile attack but not during the intervals. Diseases most likely to be confused with it: ankylostomiasis and malaria.

Of the more important trypanosome diseases of animals may be mentioned:

1. Nagana. Pathogenic for domesticated animals in South Africa. T. brucei.
2. Surra. Pathogenic for horses in India and Philippines. T. evansi.
3. Dourine. Transmitted by coitus in horses. T. equiperdum.
4. Mal de caderas. Affects horses in South America. T. equinum.

A harmless infection, especially in sewer rats, is due to T. lewisi. Transmission of this rat trypanosomiasis can apparently be brought about through the agency of both fleas and lice. In the flea there is apparently a developmental cycle of a duration of one week.

There are many trypanosomes in birds, fish, frogs, etc.

Trypanosplasma.

The genus Trypanoplasma has a rather large blepharoplast, from which arise two flagella. One extends forward as a free anterior flagellum, while the other projects posteriorly, running along the border of the undulating membrane. This genus is not known for man.

Leishmania.

The genus Leishmania includes two species: one, the L. donovani, the parasite of kala azar, and the other the L. tropica, the parasite of oriental sore. These are undoubtedly different species, inasmuch as in sections of India, where tropical ulcer was common, there was no kala azar, and in Assam where kala azar prevailed there were no Leishman-Donovan bodies to be found in smears from the tropical ulceration there present, except rarely in cases of general infection. L. tropica has been cultivated by Nicolle.

These parasites are typically intracellular, being within either polymorphonuclears, which contain only one or two of the bodies, or in large mononuclears, in which there may be as many as six. They may be packed, however, in phagocytic endothelial cells.

In smears taken during life we may find the bodies imbedded in a faintly blue staining matrix; after death and in sections of tissue such an appearance is not seen. In the spleen they are not found in the Malpighian bodies, but in the phagocytic cells lining the lymph spaces. The parasites occur in the peripheral circulation in about 80% of the cases. They abound in the liver and spleen. The parasite is oval and about $2 \times 3\mu$. There are two distinct chromatin staining masses. The larger nucleus is more or less spherical, peripherally situated, and stains faintly, while the smaller chromatin mass is generally rod-shaped and stains intensely. It has been recently recommended that instead of liver or splenic puncture for the demonstration of these bodies, a blister be raised and a smear from that containing many polymorphonuclears might show these bodies. The affection is characterized by a leukopænia so that it is very difficult to demonstrate the parasites in ordinary blood smears.

By cultivating the parasites obtained from splenic puncture in acidified sodium citrate solution at room temperature, Rogers succeeded in obtaining flagellated forms similar to Herpetomonas. An anterior flagellum proceeds directly from the blepharoplast. The bed-bug is supposed to be the intermediary host (Patton has traced developmental stages in the bed-bug).

The genera Herpetomonas and Crithidia are frequently found in the alimentary tract of insects and have caused confusion in the search for developmental forms of various pathogenic flagellates in transmitting

insects. In Herpetomonas, of which the type species is H. muscæ domesticæ, the body is spindle-shaped, while in Crithidia it is quite pointed at only one end and in Crithidia the blepharoplast is very near the nucleus.

There is no undulating membrane in either of these genera, this differentiating them from Trypanosoma.

Darling has reported from Panama a protozoon somewhat like Leishmania in which the cells of lungs, liver, spleen, and lymphatic glands contained numerous parasites about 3 to 4μ in diameter, slightly oval in outline, and containing a large and small chromatin staining mass. He has given it the name Histoplasma capsulata.

Trichomonas.

Trichomonas vaginalis.—This parasite has a fusiform body and is about 18 x 10μ. It has three flagella arising from the anterior end and an undulating membrane. It lives in vaginal mucus which has an acid reaction. A change of reaction, as at menstruation, causes them to disappear. Forms similar to the T. vaginalis have been found in the intestine and in sputum from putrid bronchitis.

These flagellates are generally considered harmless, although doubt as to this is expressed by some authors.

Lamblia.

Lamblia intestinalis.—These parasites are about 10 x 15μ and have a pear-shaped body with a depression at the blunt anterior end. This depression enables the flagellate to attach itself to the summit of an epithelial cell. Around the depression are three pairs of flagella which are constantly in motion. Another pair of flagella project from either side of the blunt little tail-like projection. When stained, the parasites have a pyriform shape with two chromatin staining areas on either side of the anterior end. When encysted, they assume a circular shape. This parasite is generally considered as of little importance, but inasmuch as, when in great numbers in the cæcum and appendix, they may give rise to symptoms resembling appendicitis and as they are responsible for a chronic and intractable diarrhœa associated with mental and physical depression, this is undoubtedly an affection only minor in importance to amœbic infection. It is a very common infection in the tropics.

INFUSORIA (CILIATA).

The Infusoria are the most highly developed of the Protozoa.

The bodies of Infusoria are oval and may be free or attached to a stalk-like contractile pedicle, as with Vorticella, or they may be sessile. The cilia, which are characteristic, may be markedly developed around the cytostome (mouth) and serve the purpose of directing food into the interior, while others act as locomotor organs. The body is enveloped by a cuticle which may only have one opening or slit, to serve as mouth; or it may have a second one, a cytopyge or anus. Usually the fæcal matter is ejected through a pore which may be visible only when in use. They usually have a large nucleus and a small one. Infusoria tend to encyst when conditions are unfavorable (as when water dries up in a pond). When the cilia are evenly distributed over the entire body of the ciliates we have the order Holotricha; when ciliated all over, but with more prominent cilia surrounding the peristome, we call the order Heterotricha. It is to this order that the Infusoria of man belong.

Balantidium coli.—This is the only ciliate of importance in man. It is a common parasite of hogs. It is from 60 to 100μ long by 50 to 70μ broad, and has a peristome at its anterior end which becomes narrow as it passes backward. It has an anus. The ectosarc and the endosarc are distinctly marked. The cuticle is longitudinally striated.

These parasites cause an affection similar to dysentery and may bring about a fatal termination. It is almost impossible to escape noticing the actively moving bodies if a fæcal examination is made. When encysted they are round.

Another ciliate, the B. minimum, 25 x 15μ, has also been reported for man.

Nyctotherus faba has a kidney-shaped body and is about 25 by 15μ. It has a large contractile vacuole at the posterior end. It has a large nucleus in the center with a small fusiform micronucleus lying close to it. It has only been reported once for man.

SPOROZOA.

This class of protozoa gets its name from the method of reproduction—sporulation. These parasites rarely show binary fission. While the sporozoa are found within cells, in the tissues and in internal cavities, as intestine and bile ducts, yet it is as inhabitants of the blood that they have their greatest importance for man—these are known as Hæmo-

DESCRIPTION OF PLATE I.

(Kolle and Wassermann.)

Malarial Parasites.

1. Two tertian parasites about thirty-six hours old, attacked blood-corpuscles magnified.

2. Tertian parasite about thirty-six hours old; stained by Romanowsky's method. The black granule in the parasite is not pigment but chromatin. Next to it and to the left is a large lymphocyte, and under it the black spot is a blood plate.

3. Tertian parasite, division form nearby is a polynuclear leukocyte.

4. Quartan parasite, ribbon form.

5. Quartan parasite, undergoing division.

6. Tropical fever parasite. (Æstivo-autumnal.) In one blood-corpuscle may be seen a smaller, medium, and large tropical fever-ring parasite.

7. Tropical fever parasite. Gametes half-moon spherical form. Smear from bone marrow.

8. Tropical fever parasite which is preparing for division heaped up in the blood capillaries of the brain.

Asexual Forms.

9. Smaller tertian ring about twelve hours old.

10. Tertian parasite about thirty-six hours old, so-called amœboid form.

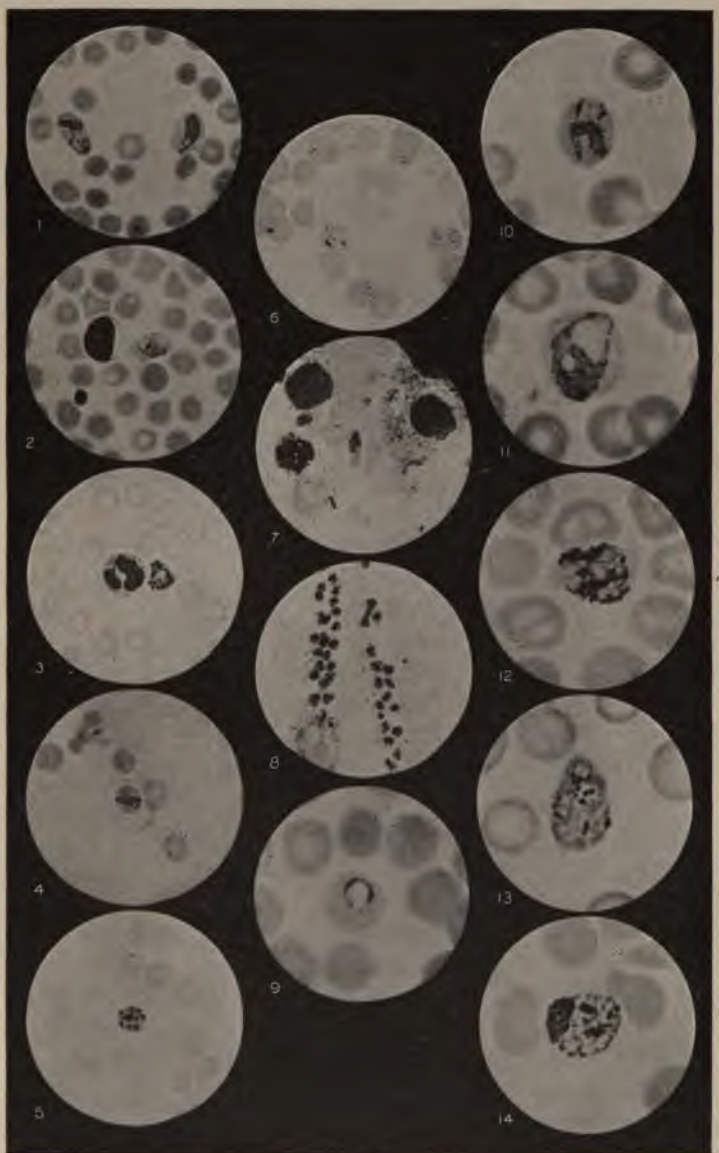
11. Tertian parasite still showing ring form forty-two hours old.

12. Tertian parasite, two hours before febrile attack. The pigment is beginning to arrange itself in streaks or lines.

13. Tertian parasite further advanced in division. Pigment collected in large quantities.

14. Further advanced in the division. (Tertian parasite.)

PLATE I.



DESCRIPTION OF PLATE II.

(Kolle and Wassermann.)

Malarial Parasites.

15. Complete division of the parasite. Typical mulberry form.

16. To the left is the completed division form, an almost developed gamete, which is to be recognized by its dispersed pigment.

17. A tertian ring parasite, small size broken up.

18. Three-fold infection with tertian parasite. The oval black granules are the chromatin granules.

19. To the left, tertian parasite with large, sharply demarked, and deeply colored chromatin granules. To the right, tertian parasite. Both thirty-six hours old. Both probably gametes.

20. Tertian parasite thirty-six hours old, ring form.

21. Tertian parasite with beginning chromatin division, with eight chromatin segments.

22. Tertian parasite chromatin division farther advanced with twelve chromatin granules, in part triangular in form.

23. Completed division figure of a tertian parasite. Twenty-two chromatin granules.

24. The young tertian parasites separating themselves from each other. The pigment remains behind in the middle.

25. Quartan ring parasite, which is hard to differentiate from large tropical ring or small tertian ring.

26. Quartan ring lengthening itself.

27. Small quartan ribbon form.

28. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

PLATE II.

Eimeria stiedæ.—This sporozoon is usually known as the Coccidium cuniculi or C. oviforme. It is most frequently found in the epithelium of the bile ducts. It has very rarely been reported for man. In these cases (about five) cysts of the liver have been found containing coccidia. The parasite is about 40 x 20μ, and is oval in shape with a double outlined integument. The sporozoites, which form inside, are falciform in shape. These escape and enter fresh epithelial cells, and thus the process of schizogony goes on. The parasites of the liver are larger than those found in the intestines, these latter being only about 30 x 15μ. In the fæces the form most often found is the oocyst, about 40 x 20μ. Infection takes place by ingestion of the oocyst.

Isospora bigemina.—This parasite, formerly called the Coccidium bigeminum, lives in the intestinal villi of dogs and cats. It is about 12 x 8μ, and shows a highly refractile envelope (oocyst) containing two biscuit-shaped spores. It has been reported for man three times.

Hæmosporidia.

Of the sporozoa found in the blood (Hæmosporidia), the **malarial parasites** are the only ones connected with disease in man.

In addition to man, infections with parasites of a similar nature are found in monkeys (Plasmodium kochi; the sexual forms alone seem to be present), in birds (Hæmamœba relicta; this organism is usually designated Proteosoma. An infection of crows and pigeons of like nature is Halteridium). Numerous hæmosporidia have been reported for bats, various other mammals, tortoises, lizards, etc.

The life history of the malarial parasite is one of the most interesting chapters in medicine. Laveran discovered the parasite in 1880. In 1885, Golgi noted that sporulation occurred simultaneously at time of malarial paroxysm. Koch, Golgi, and Celli demonstrated existence of different species for different types of fever. King and Laveran (1884) considered possibility of mosquito transmission. Manson (1894) formulated hypothesis that gametes were destined to undergo development in the mosquito from observing that flagellated bodies only appeared some time after the blood was withdrawn.

Ross (1895) demonstrated that flagellation takes place in the stomach of the mosquito. McCallum (1897) saw fertilization of macrogametes by microgametes of Halteridium. Opie recognized differences in sexual characteristics.

DESCRIPTION OF PLATE III.

(Kolle and Wassermann.)

Malarial Parasite.

29, 30, 31. The quartan ribbon increases in width. The dark places consist almost entirely of pigment,

32. Beginning division of the quartan parasite and the black spot in the middle is the collected pigment.

33. Quartan ring.

34. Double infection with quartan parasites.

35. Wide quartan band. The fine black stippling in the upper half of the parasite is pigment.

36. Beginning division of the quartan parasite. The chromatin (black fleck) is split into four parts.

37. Division advanced, quartan parasites.

38. Typical division figure of the quartan parasite.

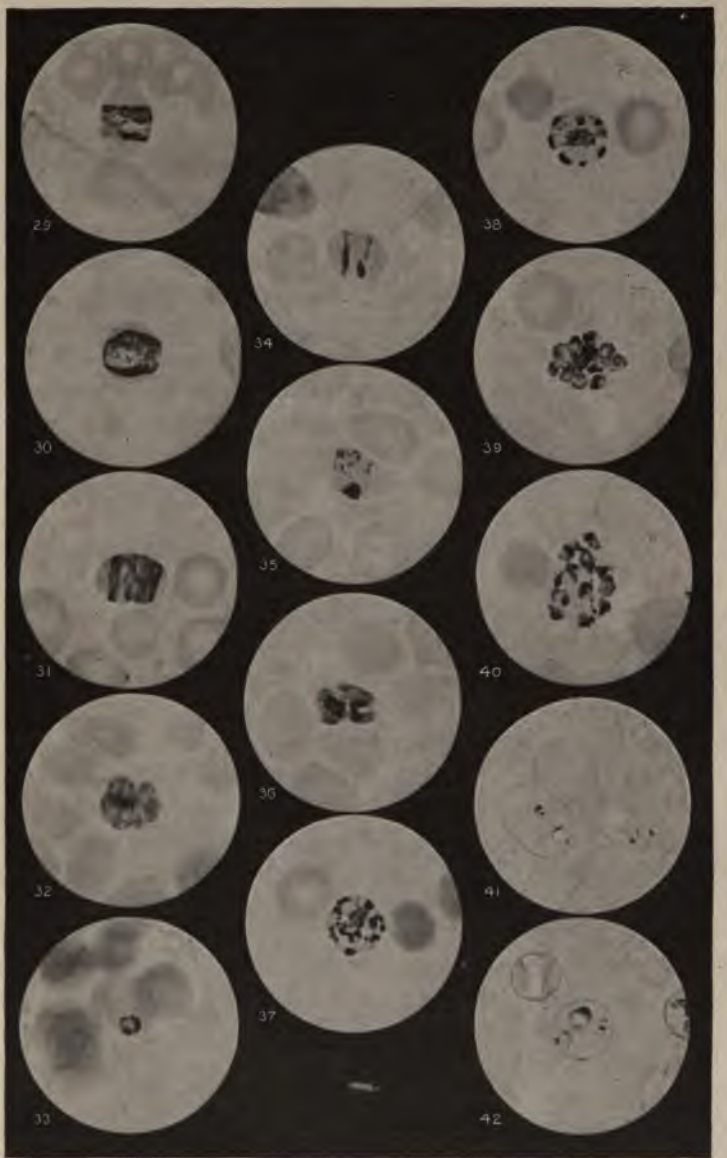
39. Finished division of the quartan parasite. Ten young parasites, pigment in the middle.

40. Young parasites separated from one another.

41. Small and medium tropical ring, the latter in a transition stage to a large tropical ring.

42. Small, medium and large tropical ring, together in one corpuscle.

PLATE III.



Ross (1898) demonstrated life cycle of bird malaria (Proteosoma), showing formation of zygotes and presence of sporozoites in salivary glands. Grassi and Bignami proved the cycle for Anophelinæ for human malaria. In 1900 (Sambon and Low), infected mosquitoes from Italy were sent to London, where, by biting, they infected two persons.

Life History.—When man is at first infected by sporozoites we have starting up a nonsexual cycle which is completed in from forty-eight to seventy-two hours, according to the species of parasite. The falciform sporozoite bores into a red cell, assumes a round shape and continues to enlarge (schizont). Approaching maturity, it shows division into a varying number of spore-like bodies. At this stage the parasite is termed a merocyte. When the merocyte ruptures, these spore-like bodies or merozoites enter a fresh red cell and develop as before.

At the time that the merocyte ruptures it is supposed that a toxin is given off which causes the malarial paroxysm. The cycle goes on by geometric progression from the first introduction of the sporozoite, but it is usually about two weeks before a sufficient number of merocytes rupture simultaneously to produce sufficient toxin for symptoms (period of incubation). This cycle is termed schizogony.

After a varying time, whether by reason of necessity for renewal of vigor of the parasite by a respite from sporulation, or whether from a stand-point of survival of the species, sexual forms (gametes) develop. Some think that sporozoites of sexual and nonsexual characteristics are injected at the same time. It is usually considered, however, that sexual forms develop from preexisting nonsexual parasites.

These gametes show two types: the one which contains more pigment, has less chromatin, and stains more deeply blue is the female—a macrogamete; the other with more chromatin, less pigment, and staining grayish-green rather than blue is the male—a microgametocyte. When the gametes are taken into the stomach of the Anophelinæ, the male cell throws off spermatozoa-like projections, which have an active lashing movement and break off from the now useless cell carrier and are thereafter termed microgametes. These fertilize the macrogametes and this body now becomes a zygote.

By a boring-like movement the zygote goes through the walls of the mosquito's stomach, stopping just under the outer epithelial layer of the stomach or mid-gut. It continues to enlarge until about the end of one week it has grown to be about 60μ in diameter and has become packed with hundreds of delicate falciform bodies.

DESCRIPTION OF PLATE IV.

(Kolle and Wassermann.)

Malarial Parasite.

43. To the left a young (spore) tropical parasite. To the right a medium and large tropical parasite.

44. An almost fully developed tropical parasite. The black granules are pigment heaps.

45. Young parasites separated from one another. Broken up division forms twenty-one new parasites.

46. To the left a red blood-corpuscle with basophilic, karyochromatophilic granules. Prototype of malarial parasite. On the right a red blood-corpuscle with remains of nucleus.

Sexual Forms or Gametes.

47. An earlier quartan gamete (macrogamete in sphere form), female.

48. An earlier quartan gamete (microgametocyte), male.

49. Tertian gamete, male form (microgametocyte).

50. Tertian gamete, female (macrogamete).

51. Tertian gamete (microgametocyte) still within a red blood-corpuscle.

52. Macrogamete tertian within a red blood-corpuscle.

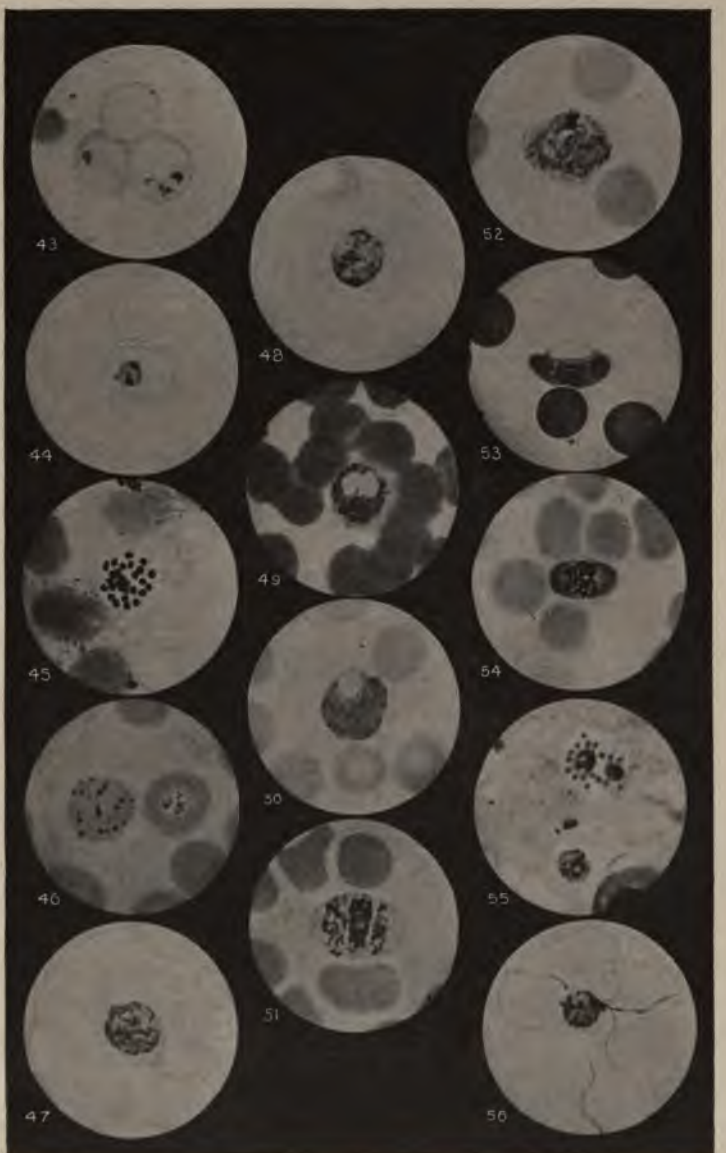
53. Tropical fever. (Æstivo-autumnal) gamete, half-moon (crescent) still lying in a red blood-corpuscle. In the middle is the pigment. The concave side of the crescent is spanned by the border of the red blood-corpuscle.

54. Gamete, tropical fever parasite.

55. Gamete of tropical fever parasite heavily pigmented.

56. Gamete of the tropical fever parasite (flagellated form), microgametocyte sending out microgametes (flagella or spermatozoa).

PLATE IV.



Zygotes of benign tertian show little rod-like particles of yellowish pigment—those of malignant tertian black clumps, which, however, are not so coarse as those of quartan.

The mature zygote now ruptures and the sporozoites are thrown off into the body cavity. They make their way to the salivary glands and thence, by way of the veneno-salivary duct in the hypopharynx, they are

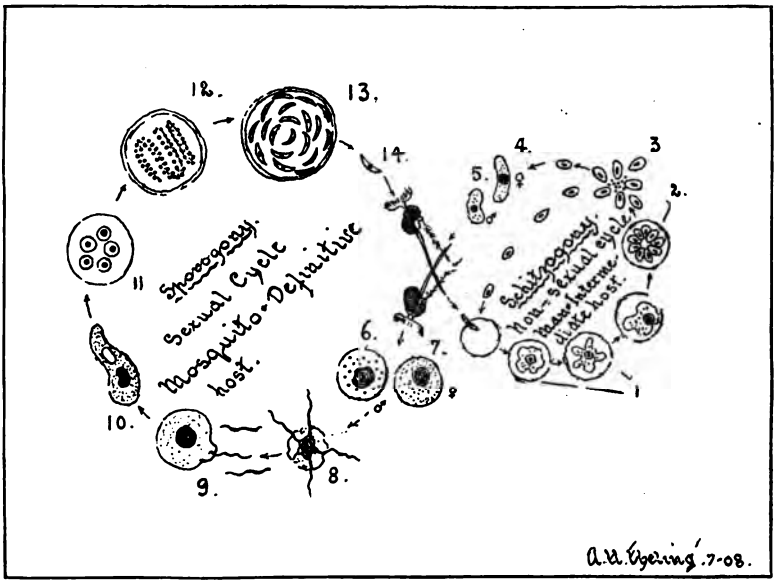


FIG. 59.—Sexual and nonsexual cycle of malaria. 1, Schizonts; 2, merocyte; 3, merozoites; 4, macrogamete; 5, microgametocyte; 6 and 7, gametes in stomach of mosquito; 8, microgametocyte throwing off microgametes; 9, microgamete fertilizing macrogamete; 10, vermiculus or zygote; 11 and 12, zygotes; 13, zygote distended with sporozoites; 14, sporozoites.

introduced into the circulation of the person bitten by the mosquito, and start a nonsexual cycle. As the sexual life takes place in the mosquito, this insect is the definitive host—man is only the intermediary host.

It must be remembered that only certain genera and species of Anophelinæ are known malaria transmitters; thus Stephens and Christophers, in dissecting 496 mosquitoes of the species M. rossi, did not find a single gland infected with sporozoites. With M. culicifacies, however, twelve in 259 showed infection.

This is one of the methods of determining the endemicity of malaria or the malarial index. There are two other methods: 1. by noting the prevalence of enlarged spleen, and 2. by determining the number of inhabitants showing malarial parasites in the blood. This index is best determined from children between two and ten years of age, as children under two years show too high a proportion of parasites in the peripheral blood while those over ten years of age show too great an incidence of enlarged spleens.

There are three species of malarial parasites: 1. the Plasmodium vivax, that of benign tertian—cycle, forty-eight hours; 2. the Plasmodium malariæ, that of quartan—cycle, seventy-two hours; and 3. the Plasmodium falciparum, that of æstivo-autumnal or malignant tertian —cycle of forty-eight hours.

Variations in cycles may be produced by infected mosquitoes biting on successive nights, so that one crop will mature and sporulate twenty-four hours before the second. This would give a quotidian type of fever. In æstivo-autumnal infections anticipation and retardation in the sporulation causes a very protracted paroxysm, lasting eighteen to thirty-six hours; this tends to give a continued or remittent fever instead of the characteristic type.

UNSTAINED SPECIMEN (FRESH BLOOD).

	P. vivax. (Benign tertian.)	P. malariæ. (Quartan.)	P. falciparum. (Malignant tertian) (Æstivo-autumnal.)
Character of the infected red cell.	Swollen and light in color.	About the size and color of a normal red cell.	Tendency to distortion of red cell rather than crenation. Shriveled appearance. (Brassy color.)
Character of young schizont.	Amœboid outline. Rarely more than one in r.c.	Has frosted glass band or ribbon-like appearance.	Small, distinctly round, crater-like dots not more than one-sixth diameter of red cell. Two to four parasites in one red cell common.
Character of mature schizont.	Amœboid with fine yellow-brown pigment which shows active swarming movement.	Rather oval in shape. Sluggish movement of peripherally placed coarse black pigment.	Only seen in overwhelming infection. Have scanty fine black pigment clumped together.

STAINED SPECIMEN.

	P. vivax. (Benign tertian.)	P. malariæ. (Quartan.).	P. falciparum. (Malignant tertian) (Æstivo-autumnal.)
Character of infected red cell.	Larger and lighter pink than normal red cell. Shows "Schüffner's dots."	About normal size and staining.	Shows distortion and some polychromatophilia and stippling. Rarely we have coarse cleft-like reddish dots—Maurer's spots.
Character of young schizont.	Chromatin mass usually single and situated in line with the ring of the irregularly outlined blue parasite.	Round rings with the chromatin mass inside of the line of the ring.	Very small sharp band-like rings, with a chromatin mass protruding outside the ring. Often appears on periphery of red cell. Frequently two chromatin dots.
Character of mature schizont.	Fine pigment rather evenly distributed in irregularly outlined parasite.	Coarse pigment rather peripherally arranged in an oval parasite.	Very rarely seen in peripheral circulation in ordinary infection. Pigment clumps early.
Character of merocyte.	Irregular division into fifteen or more spore-like chromatin dot segments.	Rather regular division into eight or ten merozoites—Daisy.	Sporulation occurs in spleen, brain, etc. Rarely in peripheral circulation. Eight to ten chromatin staining merozoites.
Character of macrogamete.	Round deep blue, abundant, rather coarse pigment, chromatin at periphery.	Round, similar to P. vivax but smaller.	Crescentic, deep blue, pigment clumped at center, chromatin scanty and in center.
Character of microgametocyte.	Round, light green-blue, pigment less abundant, chromatin abundant and located centrally or in a band.	Round like P. vivax.	More sausage-shaped than crescent. Light blue. Pigment scattered throughout. Chromatin scattered.

In parthenogenesis, as observed in P. vivax, the nonsexual forms and the males die off leaving only the female forms. The nucleus divides into a dense and light portion. The latter degenerates and the former goes on to merozoite formation.

This is Schaudinn's explanation of relapses. Another explanation of latent malaria is by conjugation of two ring forms.

In the diagnosis of malaria one should always examine both a fresh specimen and a stained one, as each method gives valuable information in differentiating species. When time will not permit the examination by both methods, always use the smear stained by Wright's stain, as the small peripherally situated rings of æstivo-autumnal fever may escape notice in a fresh specimen.

SARCOSPORIDIA.

Sarcosporidia are sporozoa found in the striped muscles of various mammals and birds. They are common in the pig and mouse and have been reported for man in three well authenticated cases. In the last, Darling found these protozoa in the biceps muscle of a negro patient in Panama. In Baraban's case the laryngeal muscles at autopsy were found to show cysts about 1/15 inch long which contained sickle-shape sporozoites about 9μ long.

They are known also as Miescher's tubes when in muscle fibers. They are divided into three genera: Miescheria and Sarcocystis when parasitic in muscle fiber; Balbiania, when parasitic in the intervening connective tissue of the muscles.

The method of transmission is unknown. In some places more than 50% of the sheep and pigs may show infection.

In addition to the protozoa previously referred to, there are certain infections which are considered by certain authors to be protozoal in nature.

Cytorrhyctes vaccinæ.—These parasites develop within the epithelial cells of stratified epithelium. In vaccinia, Councilman and his colleagues consider that the development only takes place in the cytoplasm of the cell. In variola, however, the developmental cycle affects the nucleus.

Cytorrhyctes luis, reported as the cause of syphilis, sporulates in the blood-vessels and in the connective tissue, not in epithelial cells.

Cytorrhyctes scarlatinæ was reported by Mallory to have been found in the skin in four cases of scarlet fever.

CHAPTER XVII.

FLAT WORMS.

CLASSIFICATION OF THE PLATYHELMINTHES (FLAT WORMS).

Class	Family	Genus	Species
Trematoda	Fasciolidæ	Fasciola	F. hepatica
		Fascioletta	F. ilocana
		Fasciolopsis	F. buski
		Dicrocœlium	D. lanceatum
		Paragonimus	P. westermanii
		Opisthorchis	O. felineus
		Clonorchis	C. sinensis
			C. endemicus
	Paramphistomidæ	Heterophyes	H. heterophyes
		Cladorchis	C. watsoni
		Gastrodiscus	G. hominis
	Schistosomidæ	Schistosomum	S. hæmatobium
			S. japonicum
			S. mansoni
Cestoda	Dibothriocephalidæ	Dibothriocephalus	D. latus
	Tæniidæ	Dipylidium	D. caninum
		Hymenolepis	H. nana
			H. diminuta
		Tænia	T. solium
			T. saginata
		Davainea	D. madagascariensis

NOTE.—Two larval Tæniidæ are found in man (Cysticercus cellulosæ and Echinococcus polymorphus).
Also two larval Dibothriocephalidæ (Sparganum mansoni and Sparganum prolifer).

TREMATODES OR FLUKES.

Flukes are generally leaf-like in outline, rarely cylindrical, and exhibit marked variation in size and shape. They are nonsegmented and do not have cilia on ectoderm. Very characteristic of them is the possession of suckers by which they hold on to the skin or alimentary system of their host.

They are divided into two orders: 1. the Monogenea in which the egg gives rise to a larva which later becomes the adult and 2. the Digenea. It is to this latter that the flukes parasitic in man belong. This order is characterized by the fact that the larva becomes parasitic in some

second animal and then gives rise to a second generation of larvæ which latter develop into adults.

The largest human fluke, Fasciolopsis buski, is from 2 to 3 inches in length, while the Heterophyes heterophyes is less than 1/12 of an inch in length. The most important fluke, the liver fluke, Clonorchis endemicus, is flat and almost transparent, while the almost equally important lung fluke, the Paragonimus westermanii, is oval, almost round and reddish-brown in color. With the exception of the Schistosomidæ, all flukes are hermaphrodites, and, with the exception of this family, all flukes have operculated eggs. The only other operculated (with a lid) eggs we meet with in man are those of the Dibothriocephalidæ.

The three important families of flukes parasitic for man are: 1. Paramphistomidæ—flukes with two suckers situated at either extremity. 2. Fasciolidæ—flukes with two suckers, one terminal, the other adjacent to it and situated ventrally. This family includes the important genera Fasciola, Opisthorchis, Dicrocœlium, Fasciolopsis, and Paragonimus. In Paragonimus the genital pore is posterior to the acetabulum, in the other genera it is anterior. Fasciola has a dendritic intestina canal which is not the case with Opisthorchis and Dicrocœlium. In Dicrocœlium the testicles are anterior to the uterus, in Opisthorchis they are posterior. 3. Schistosomidæ: In this family we have a leaf-like male which by a folding in of its sides makes a channel for the thread-like female. The sexes are separate, not hermaphroditic as with the Fasciolidæ and Paramaphistomidæ.

Flukes have two suckers which, except in the Paramphistomidæ, are quite near each other—one is termed the oral sucker and the other the ventral sucker or acetabulum. The intestinal tract consists of a pharynx, proceeding from the oral sucker, which bifurcates and terminates in blind intestinal cæca.

At the posterior extremity is an excretory pore which is at the termination of a duct which divides into ramifying branches. This is the water-vascular system. The testes, of various shapes and relations to the uterus, are more or less centrally situated and have vasa deferentia. In some flukes the receptaculum seminis is a conspicuous organ. The vitellaria are bilateral branching glands which pour nutrient material into the ootype. It is in the ootype that the eggs are formed, and opening into it we have the adjacent ovary. The shell gland is near the ovary.

A canal, known as Laurer's canal, leads from the ootype to the exterior, the function of which is in question. It is probable that as trematodes have no spermatheca, the spermatozoa from other flukes enter by way of this canal. The life history of the important human flukes is unknown. It is supposed that this, in a measure, may resemble that of the common liver-fluke of sheep (sheep rot). In this the eggs containing a ciliated embryo (miracidium) pass out in the fæces. This embryo is hatched out and, gaining the water, swims about actively until it reaches some suitable mollusk or crustacean (Limnæa truncatula). By means of a pointed end, it bores its way into the body of the gasteropod and in the pulmonary chamber becomes a bag-like structure (the sporocyst) from the germinal cells of which develop a creature with an alimentary canal (redia). The rediæ tend to break out of the sporocyst and wander to the liver of the snail. These rediæ may give rise to a second generation of rediæ.

From the rediæ minute little worms resembling adult flukes in possessing suckers, but differing in the possession of a tail, develop (cercaria). Having reached maturity, these cercariæ leave the rediæ, and, as in case of Fasciola hepatica, lose the tail, become encysted on blades of grass, to be eaten by sheep and again commence the cycle. The encysted cercariæ develop into adult liver flukes. It is probable that with many flukes the cercariæ enter some host, as mollusk, insect, or fish, and that it is by eating such animals as food that man becomes infected. Looss thinks it possible that the miracidium of Schistosomum hæmatobium may bore its way directly into man, as do the larvæ of the hookworm. Manson also suggests that the reporting by Musgrave of 100 mature lung flukes in a psoas abscess makes it very probable that these parasites entered the body as miracidia. The idea in China is that the infection with the common liver fluke of man is brought about by eating fish. Fluke disease is generally known as distomatosis or distomiasis.

Liver Flukes.

Fasciola hepatica.—This fluke, while of enormous conomic importance by reason of destruction of sheep, has only been reported twenty-three times in man, and in these instances does not seem to have occasioned marked symptoms. There is, however, a possible importance of F. hepatica in connection with a peculiar affection known as

"halzoun." This results from the eating of raw goat-liver, and it is supposed that the flukes crawl up from the stomach and, entering the larynx or attaching themselves about the glottis, produce the asphyxia characteristic of the disease.

Dicrocœlium lanceatum.—This has only been reported seven times in man. The symptoms are unimportant. The fluke is about 1/3 of an inch long, with testicles anterior to the uterus.

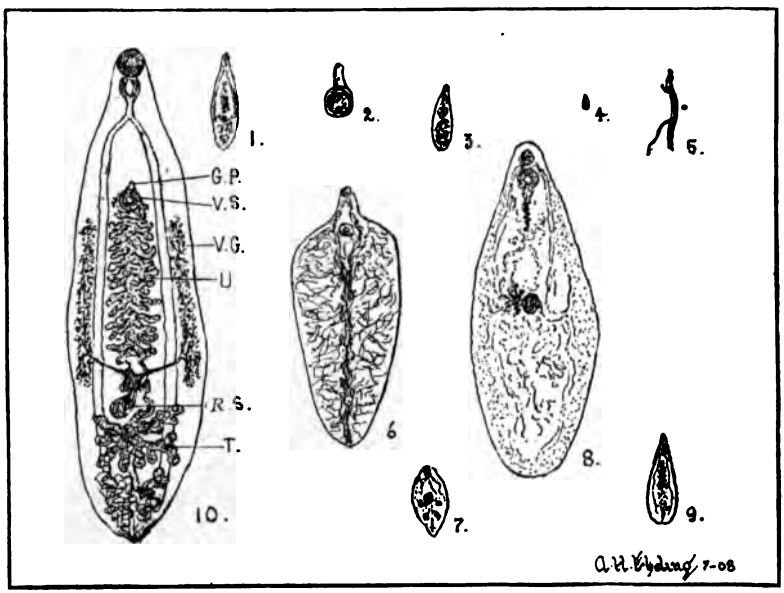


FIG. 60.—Trematodes of man, natural size. 1, Clonorchis endemicus (Opisthorchis sinensis); 2, Gastrodiscus hominis; 3, Dicrocœlium lanceatum; 4, Heterophyes heterophyes; 5, Schistosomum hæmatobium; 6, Fasciola hepatica; 7, Paragonimus westermanii; 8, Fasciolopsis buski; 9, Opisthorchis felineus; 10, anatomy of C. endemicus (enlarged). G. P., genital pore; V. S., ventral sucker; V. G., vitelline glands; R. S., receptaculum seminis; T., branched testicles.

Clonorchis endemicus.—This fluke and the C. sinensis are the most important of the human liver flukes. Until recently these flukes were known as Opisthorchis sinensis. Looss has separated this genus from Opisthorchis principally by the characteristic of branching testicles—those of Opisthorchis being lobed. This fluke is very common *in China* and Japan—in certain sections of Japan 20% of the population

being infected. This fluke is about 3/4 of an inch long and 1/6 of an inch broad. When squeezed out of the thickened bile ducts it is so transparent and glairy as almost to resemble glairy mucus. This fluke is supposed to produce most serious symptoms, as indigestion, swelling and tenderness of liver, ascites, œdema, and a fatal cachexia. As a matter of fact, many physicians in China attribute very little pathogenic importance to it. The disease is diagnosed by the presence of the ova in the stools.

Opisthorchis felineus.—This fluke is smaller than the C. endemicus, and is a common parasite of the gall bladder and bile ducts of cats. There are two lobed testicles in this species instead of dendritic ones as in C. endemicus. In certain parts of Siberia the parasite is found in more than 6% of the human autopsies. The symptoms are similar to those caused by C. endemicus.

Other liver flukes of less importance which have been reported for man are: 1. Opisthorchis noverca. This was found in bile ducts of two natives of Calcutta. It was lancet-shaped and covered with spines.

2. Metorchis truncatus: This is a small fluke, 1/12 inch long, squarely cut across at its posterior end and covered with spines. This was possibly found once in man.

Intestinal Flukes.

Cladorchis watsoni.—This fluke is about 1/3 of an inch long, of oval outline but broader at posterior end and has an indistinct oral sucker and a large sucker at the other end. This parasite has only been reported once. Eggs, 125 x 75μ.

Gastrodiscus hominis.—This fluke is about 1/4 of an inch long and has a disk about 1/6 of an inch in diameter from which proceeds a teat-like projection, bearing an oral sucker. While it has only been reported twice for man, indications are that it is probably fairly common in India and Assam. Eggs, 150 x 72μ.

Fasciolopsis buski.—This is probably a rather common parasite in India, as Dobson found the eggs in 1% of the stools of more than 1000 coolies. The fluke is from 2 to 3 inches in length and about 1/2 of an inch in breadth. It is thick, brown in color, and has a very large acetabulum, three times the size of the oral sucker. The branched ovary and shell gland lie in the center with the branched testicles posterior. Eggs, 125

x 75μ. These parasites cause dyspeptic symptoms and an irregular diarrhœa. It is also called Distomum crassum.

Heterophyes heterophyes.—This exceedingly small fluke, which can be recognized by its small size (less than 1/12 of an inch long) and large, prominent acetabulum, was formerly supposed to be rare. The

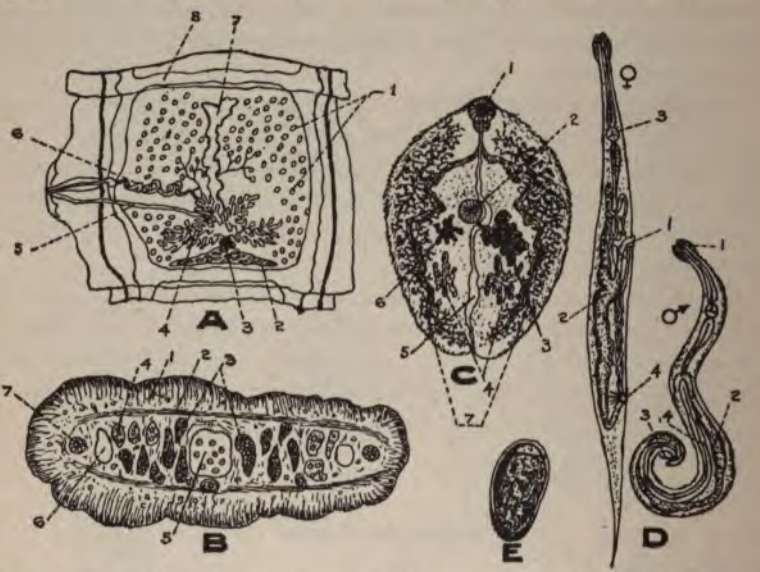


FIG. 61.—Anatomy of a tape-worm, Tænia solium (A., longitudinal, B., cross section); a fluke, Paragonimus westermanii (C.); male and female nematode, Oxyuris vermicularis (D.).

A. 1, Testes; 2, yolk glands; 3, shell glands; 4, ovary; 5, vagina; 6, vas deferens; 7, uterus before branching; 8, water-vascular system.

B. 1, Cuticle; 2, circular muscle; 3, ovary; 4, testes; 5, uterus; 6, excretory canal; 7, nerve cord.

C. 1, Oral sucker; 2, acetabulum; 3, uterus; 4, testes; 5, excretory canal; 6, ovary; 7, yolk glands.

D. (a) Female. 1, Vulva; 2, uterus; 3, bulb of œsophagus; 4, anus. (b) Male. 1, Bulbous mouth end; 2, testes; 3, spicule; 4, alimentary canal.

E. Egg of P. westermanii.

oral sucker is much smaller than the acetabulum. The elliptical testicles lie at the extreme posterior end. The eggs are 30 x 17μ. Very characteristic of this genus is the large sucker-like genital pore just below and to one side of the acetabulum. Looss has shown that it is quite common in Egypt, he having found it twice in Alexandria in nine autopsies. The parasites occupy the ileum. It is common in dogs.

Fascioletta ilocana.—This is a small fluke, about 1/4 inch long. There are two massive testicles in the posterior part of body. The acetabulum is prominent. The egg of this small fluke is quite large (100μ) and has an operculum. These trematodes were found by Garrison in five natives of Luzon, P. I., after treatment with male fern.

Lung Flukes.

Paragonimus Westermanii.—In certain parts of Japan and Formosa it is estimated that as many as 10% of the inhabitants may harbor this parasite.

It is also common in China, and recently many cases have been reported in the Philippines. Dr. Stiles states that around Cincinnati, Ohio, there is quite a heavy infection among the hogs, so that it may be that certain cases diagnosed in man as pulmonary tuberculosis are paragonimiasis.

It is popularly known as endemic hæmoptysis on account of the accompanying symptoms of chronic cough and expectoration of a rusty-brown sputum. After violent exertion, and at times without manifest reason, attacks of hæmoptysis of varying degrees of severity come on. The characteristic ova are constant in the sputum and establish the diagnosis. The fluke itself is a little more than 1/3 of an inch long and is almost round on transverse section, there being, however, some flattening of the ventral surface. The acetabulum is conspicuous and opens just anterior to the middle of the ventral surface. Eggs about 90 x 65μ.

The branched testicles are posterior to the laterally placed uterus and the genital pore opens below the acetabulum. The branched ovary is opposite the uterus on the other side.

It is rather flesh-like in appearance and is covered with scale-like spines. The flukes are usually found in tunnels in the lungs, the walls of which are of thickened connective tissue. There may be also cysts formed from the breaking down of adjacent tunnel walls. In addition to lung infection with this fluke, brain, liver, and intestinal infections may be found. Musgrave was the first one to call attention to the frequency of general infection with this parasite (paragonimiasis) in the Philippines. He found it in seventeen cases in one year. The life history, beyond the stage of miracidium, is unknown.

Another fluke which has been reported from the lung is Fasciola

gigantea (very similar to F. hepatica). This was coughed up by a French officer who had been in Africa.

Blood Flukes.

Schistosomum hæmatobium.—Flukes of the circulatory system are of great importance in Egypt, South Africa, Japan, and the West Indies. The disease is named bilharziasis after Bilharz who first associated the parasite and the disease. It seems probable that there are at least three human species, differentiated principally by the character of the egg. In the blood-fluke disease of Egypt, (S. hæmatobium), the parasite chiefly infects the bladder and the egg has a terminal spine. The lateral-spined ovum is also found in the fæces. In the West Indies, as shown by the reports of Surgeon Holcomb from Porto Rico, rectal bilharziasis is rather common. In these cases the egg is practically always lateral-spined. The adults of this species, the S. mansoni, are scarcely, if at all, to be distinguished from the (S. hæmatobium). With S. japonicum, the name of the Eastern species, there is not only the difference that the eggs are without spines, but, in addition, the skin of the adult parasite is not tuberculated, as is the case with the other two species. It is slightly smaller, the acetabulum projects more prominently, and the lower part of the male infolds more markedly than in S. hæmatobium. Catto considers that the S. japonicum may live in both arteries and veins. The other two species only live in branches of the portal vein. The blood flukes are about 1/2 inch long. All of these flukes live separately until maturity. At this time the female enters what is known as the gynæcophoric canal of the male; this canal is formed by the infolding of the sides of the flat male fluke, thus giving a rounded appearance to the male. The female is longer than the male (about 5/6 of an inch long), and is thread-like and of a darker color. Her two extremities project from the canal of the male in which she lives.

The oral sucker of the male is infundibuliform and is smaller than the pedunculated acetabulum. In the female the oral sucker is larger than the acetabulum. The eggs are fusiform, yellowish in color, have a thin shell and a terminal spine.

The most prominent symptoms of the Bilharz disease are hæmaturia and bladder irritation; later on calculus formation. In rectal *bilharziasis the* symptoms are more those of bleeding piles or of a mild

dysentery. In the Japanese infection the symptoms point more to liver and spleen, there being ascites, cachexia, and a bloody diarrhœa.

The eggs of the S. japonicum are readily found in the fæces; they are about 100 x 70μ. They are oval, transparent, and with a smooth shell, within which can be made out the outlines of an embryo. Upon adding water the ciliated embryo begins to show movement in about ten minutes and shortly afterward bursts out of the shell and swims about actively. It is more melon-shaped than the miracidium of S. hæmatobium.

The life history is not known of any of these flukes. Looss conjectures that it is probable that the miracidium enters the skin, not requiring an intermediary host. Frequent experiments have failed to show any mollusk, etc., which attracted the embryo. Evidence seems to show that those who are constantly wading about in the water of the pools or the mud of the fields are the ones most subjected to infection.

Katsurada, by experiments with a cat and dog, has proved that infection will take place through the shaved skin of an animal held in infected water—none of the water being allowed to enter by mouth. Fully developed miracidia and male and female flukes were found in the portal vein. It is thought that further development of the miracidia in the body may account for the heavy infection.

Turner has recently noted the frequency of bilharzial affections of the lungs in South Africa (50% in natives) and he thinks this may be an important factor in the prevalence of lung diseases in the natives of this region. He considers bathing in contaminated waters of prime importance in the causation of the infection which he thinks is probably by way of the skin.

A recent view is that the miracidium enters while bathing by the preputial channel, hence the value of circumcision.

If urine containing eggs is diluted with water the miracidium breaks out of the shell and swims about as if in search of some desired object.

The view is also entertained that the miracidium may gain access to the body through the drinking water; there is much evidence against this. However access to the body is gained, it is known that the larval forms make their way to the liver where they develop. Arriving at maturity, the males and females become united and proceed to the terminal branches of the portal vein, where the irritating eggs, given off by the female, give rise to the symptoms.

CESTODE OR TAPE-WORM INFECTIONS.

The cestodes and trematodes constitute the two great divisions of the flat worms. Anatomically, a tape-worm may be considered as a series of individual flukes united in one ribbon-like colony. The cestode segments, or proglottides are covered by an elastic cuticle and in their interior usually contain striated elliptical bodies composed of calcium carbonate about 5 to 25μ according to the species in which they are found.

These calcareous bodies are characteristic of cestode tissue. They have been mistaken for coccidia. There is no mouth or alimentary canal in tape-worms, the segments absorbing their nourishment through the general surface.

A tape-worm is divided into the segment-producing controlling head and the series of segments or proglottides together known as the strobila. The head and neck together form the scolex. Tape-worm heads are provided with suctorial or hook-like organs, or both, to enable them to hold on to the intestinal mucosa. The importance of the head is generally recognized by the well-known fact that the permanent evacuation of one of these parasites is only arrived at when the head as well as the segments is expelled. Otherwise, additional segments will be produced. Even in tape-worms twenty-five to thirty feet in length, the head is no larger than a small shot. It carries the suckers or hooklets which best enable us to differentiate the different species. The segments adjacent to the head are immature—the sexually-mature ones being found from the middle of the body onward. The sexually-mature segment possesses a varying number of testicles: three in Hymenolepis nana and as many as 2000 in Tænia saginata. As with the flukes, they also have vasa deferentia, cirrus, ovaries, yolk glands, uterus, genital pore, etc. The location of the genital pore and the character of the branching of the uterus are of the greatest importance in differentiation. The sexually-mature proglottides may either expel their ova, when these would be found in the fæces or, as is common, they break off and pass out themselves in the fæces. Then they either expel the eggs or may be eaten by some animal and in this way effect an entrance for their ova. It is an important practical point that the fæces of a patient with T. solium or T. saginata may not show any ova, these passing out in the intact segments. The oval operculated eggs of Dibothriocephalus latus, however, are constantly in the fæces.

The "hexacanth" or six-hooked embryo, also called the onchosphere, is the essential part of the egg. The egg shell is dissolved off in the alimentary canal of the animal ingesting it, and the onchosphere bores its way through the gut to later become encysted in various tissues. In some tape-worms a ciliated embryo is liberated from the egg shell and swims about actively to enter some fish or other animal. When the six-hooked embryo reaches its proper tissue, the hooklets are discarded

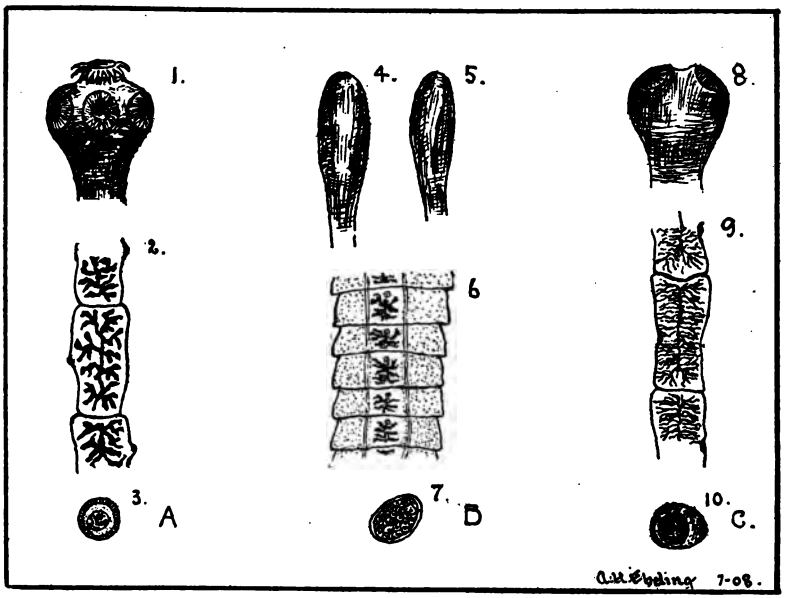


FIG. 62.—Tape-worms. A. 1, 2 and 3, Scolex, proglottides and ovum of Tænia solium; B. 4, 5, 6 and 7, Scolex, proglottides and ovum of Dibothriocephalus latus; C. 8, 9, and 10, Scolex, proglottides and ovum of Tænia saginata.
The onchosphere in 10 is shown within the outer yelk coating (frequently seen in stools). In 3 only the onchosphere within the embryonal shell is shown.

and a scolex similar to the parent one is developed. At this time we have a bladder-like structure with the scolex inverted in it. This is termed the proscolex stage. This little cyst with its scolex when ingested by another animal is digested, and the scolex, establishing itself in the intestine, develops a series of segments. The ciliated embryo of the D. latus does not form a cyst, but instead a worm-like creature similar to the adult. This is termed a Plerocercoid.

If the larval stage shows a single cyst and a single head, it is termed Cysticercus; if multiple cysts but only one head to each cyst, Cœnurus; while with multiple cysts and multiple heads in each cyst the term Echinococcus is used.

Where there is very little fluid in the cyst and the larva is of minute size, as with the Hymenolepis, the term Cercocystis is employed.

TÆNIIDÆ INFECTIONS.

Tænia saginata.—This very widely distributed tape-worm is often termed the unarmed tape-worm, to distinguish it from the T. solium or armed tape-worm. It is from 10 to 25 feet long and has several hundred proglottides. The small pear-shaped head has four pigmented elliptical suckers and no hooklets. The segments are plumper than those of T. solium, hence the name saginata. The single lateral genital pore projects markedly and in a series of segments presents, as a rule, first on one side, and then on the opposite side of the next segment (alternating). The best way to distinguish a segment of the T. saginata from the T. solium is by counting the number of lateral uterine branches; these number fifteen to thirty and branch dichotomously. The lateral divisions of the uterus of the T. solium are tree-like in their branching and only number five to twelve on each side. The ox is the intermediate host. The six-hooked embryo, having worked its way from the alimentary canal to the muscles or liver of the ox, becomes encysted (Cysticercus bovis). This little bladder-like structure is about 1/4 by 1/3 inches. Being ingested by man's eating raw or imperfectly cooked meat, the adult stage becomes established in his alimentary canal.

It is probable that the various raw-meat cures have made the infection more common. In Abyssinia the infection is said to be universal, and a man without a tape-worm to be a freak. An important point is the fact that the larval stage almost never appears in man. It is this fact which makes it a so much less dangerous parasite than the T. solium, which readily establishes a larval existence in man if the ova are introduced into the human stomach. Cooking meat always destroys the Cysticercus. A period of about two months elapses after the ingestion of the Cysticercus before the mature segments pass out of the rectum. These not only make their exit with the fæces, but are also capable of *wandering out* at other times. In this they differ from the segments of

T. solium. T. saginata next to Hymenolepis nana is the common tape-worm of the United States. Dr. Stiles has examined several hundred tape-worms during the past few years and has found only one T. solium.

Abnormalities of the scolex and proglottides are not uncommon with T. saginata. This is less frequently the case with T. solium.

Tænia solium.—The measly-pork tape-worm is smaller than the T. saginata and differs from it in having a globular head, with a rostellum, which is crowned by twenty-six to twenty-eight hooklets. The segments have only five to ten branches and are expelled only at the time of defecation. The segments or the ova having been ingested by a hog, the six-hooked embryo is liberated and becomes encysted chiefly in the tongue, neck, and shoulder muscles of the hog, as an invaginated scolex. Pork containing this Cysticercus (Cysticercus cellulosæ) is known as measly pork. If one by chance should carry the egg on his fingers to his mouth, as the result of examining mature segments, the larval stage may be established in man. If this infection is not heavy, very few symptoms may be observed. The Cysticercus, however, tends to invade the brain, next in frequency the eye, and so causes convulsions, death or blindness. Instead of only being the size of a pea, these cysts, when forming in the brain, may be the size of a walnut or larger. T. solium is comparatively common in North Germany, but is exceedingly rare in England and the United States.

Tænia africana.—This is an unarmed tape-worm, only about 5 feet long. It was found in a native soldier in German East Africa.

Hymenolepis nana.—This is generally known as the dwarf tape-worm—it is the smallest of the human tape-worms. It is from 1/4 of an inch to 1/2 inch in length, and is less than 1/25 of an inch in breadth. The genus Hymenolepis has lateral genital pores, all of which are on the same side. The head has four suckers and a rostellum, which is usually invaginated. The rostellum has twenty-four to thirty hooklets encircling it. The eggs of this species are quite characteristic, there being two distinct membranes. The inner one has two distinct knobs, from which thread-like filaments proceed. The eggs of the H. diminuta have a thicker, striated, outer membrane and there are no filaments. The eggs of the Dipylidium caninum are similar, but are found in the fæces in aggregations—several eggs in a packet. The dwarf tape-worm has been found to be the most common tape-worm in the United States. Dr. Stiles found it in about 5% of children in a Washington orphanage.

It has been estimated that in certain parts of Italy 10% of the children may be infected. The symptoms, especially nervous ones, may be marked in this infection. It has been incriminated as a cause of chyluria. Although very small, yet the number of parasites may be very great, even more than 1000. A form found in rats, which may be identical with H. nana, does not require an intermediate host. The six-hooked embryo bores into the intestinal villus and there develops a Cercocystis (larva of small dimensions with but little fluid). When fully developed, it drops into the lumen of the gut, and a new parasite is added to the already existing number of parasites. This explains the heavy infection. H. diminuta and H. lanceolata have also been reported for man a few times.

H. diminuta is much larger than H. nana, being about 10 inches long. The suckers are small and the rostellum insignificant and unarmed. The intermediate host is some insect, as a moth; the definitive, the rat. As man is not liable to eat the insect hosts the infection is rare in man.

H. lanceolata is common in geese and ducks.

Dipylidium caninum.—This is a common parasite of dogs and cats. The larval stage is passed in lice and fleas. The cases of human infection have been principally in children, probably from getting dog lice or fleas in their mouths. The head has four suckers and a rostellum, which has three or four rows of encircling hooklets. The segments have the shape of melon seeds and have bilateral genital pores.

Davainea madagascariensis.—This tape-worm has been found in Siam and Mauritius. It is about 10 inches long. The head has four suckers and a rostellum with ninety hooklets. The suckers have rings of hooklets. The genital pores are unilateral. The cockroach is supposed to be the intermediate host.

Dibothriocephalidæ Infections.

Dibothriocephalus latus.—This is frequently termed the broad Russian tape-worm. It has a small olive-shaped head with two deep winding suctorial grooves on each side; it has neither rostellum nor hooklets. The segments are quite broad, being about 1/2 by 1/5 inch At the end of the strobila they are more nearly square. The segments are very numerous, 3000 or more. The fully developed worm is about *30 feet long*. The uterus in each segment is rosette-shaped and the geni-

tal pore is ventrally situated. The eggs of this species have an operculum and a ciliated embryo. This ciliated embryo swims around and either enters some fish, especially pike, directly or through an as yet unknown intermediary. This parasite produces an intense anæmia similar to pernicious anæmia. It is a frequent parasite in Switzerland, Bavaria, Japan, Scandinavia, and Russia. Recently several cases have been reported from our Northwest, and some of the fish of the waters of that region are said to be infected. The larva is a plerocercoid and is about 1 inch long. It is said that salting, smoking, or other ordinary methods of preserving fish will not kill it.

A tape-worm, Diplogonoporus grandis has been reported from Japan. In this there are two complete sets of genital organs to each segment.

Somatic Tæniasis.

While rarely we may have the larval stage of T. solium present in man, and while certain bothriocephalid larvæ (Sparganum mansoni and Sparganum proliferum) infect man, yet they are unimportant as compared with the larval stage of the **Tænia echinoccocus**. The adult stage of this parasite is passed in dogs. It is one of the smallest tapeworms known, being only about 1/6 inch long. It has a head with four suckers and a rostellum encircled with hooks. There are only three to four segments. The larval stage, on the contrary, gives one of the largest of larval cestodes. In man it may reach the size of a child's head. The larval stage is also found in hogs and sheep, and it is probable that by reason of the dog's eating the echinococcus cyst of such animals at the abattoir we owe the increase in this serious infection.

Man contracts the infection from association with dogs. The disease is peculiarly prevalent in Iceland. As stated above, the adult stage is passed in the intestine of the dog. Should the egg-bearing segments passed by the dog contaminate the hands of man and a single egg be ingested, we may have hundreds of Tænia larvæ produced. The six-hooked embryo, leaving its shell, bores its way through the walls of the alimentary tract and especially seeks the liver, just as the embryo of T. solium seeks the brain and eye.

In the development of the cyst, after the embryo has come to rest at some point in the liver, we have formed at first an indistinctly laminated

external envelope with coarsely granular fluid contents. Later on the contents become transparent, and two distinct layers can be observed: 1. The external, markedly laminated one, and 2. the internal one, made up of small cells externally and large cells and calcareous corpuscles internally. This internal lining membrane is known as the parenchymatous or germinal layer. When the external layer is incised it curls

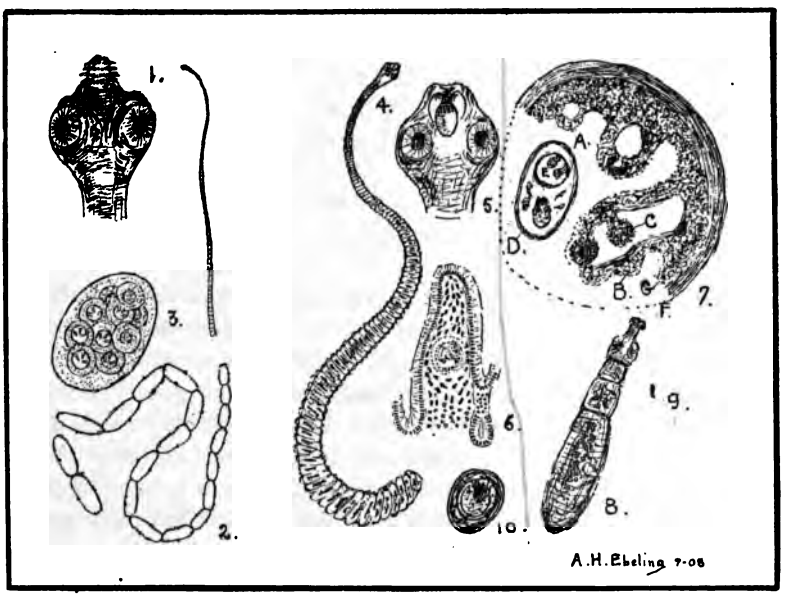


FIG. 63.—Tape-worms. 1, 2, and 3, Head, melon-shaped segments and egg packet of Dipylidium caninum; 4, 5, 6 and 10, entire worm magnified, head, larval stage in intestinal villus and ovum of Hymenolepis nana; 7, echinococcus cyst; A, mother cyst; D, daughter cyst; E, granddaughter cyst; C, scolex in brood capsule; B, brood capsule; G, parenchymatous layer; F, laminated layer; 8 and 9, Tænia echinococcus; 9, natural size.

up by reason of its elasticity. This is characteristic of such a cyst. In addition, we have an enveloping connective-tissue capsule formed by the surrounding liver substance. From the germinal layer arise the brood capsules and the scolices. In these brood capsules we have the cellular layer external—just the reverse of the mother cyst. Scolices may develop either on the outside or inside of these brood capsules. It *is interesting* to note that one onchosphere may develop hundreds of

scolices. From the parenchymatous layer of the mother cyst, daughter cysts are formed; these have an external stratified layer and an internal parenchymatous one; within them a varying number of scolices may develop. From these daughter cysts, granddaughter cysts may arise—all within the mother cyst—and hence are termed endogenous.

At times the daughter cysts work their way external to the mother cyst and proceed to develop in a manner similar to the endogenous formation. The exogenous development is rare in man, but common in hogs. Hydatids containing no scolices are called sterile. These cysts may be as large as a child's head, but are usually smaller. The fluid of these cysts contains about 1% of NaCl, also a trace of sugar; in addition there is a toxin which produces urticaria and acts as a cardiac depressant. If any quantity should escape into the peritoneal cavity at operation, it may cause death. Hydatids develop very slowly, and the duration of the disease is usually from two to eight years.

Echinococcus multilocularis is possibly due to a species different from T. echinococcus. In this we have a honeycomb arrangement with cavities filled with a gelatinous material. The majority of these cysts are without scolices. This form of hydatid is very fatal.

Sparganum mansoni.—This is a larval bothriocephalid which is about 5 to 10 inches long and has been reported ten times in Japan. It has been found in various parts of the body, as in pleural cavity, tissues about kidney, and in abscess of the thigh. They have been found in the urethra and under the conjunctiva. They resemble ribbon-like strings of fat.

Sparganum prolifer.—This has been reported from Japan as a larval form in the subcutaneous tissue. Stiles has found these larval forms in skin lesions in Florida. They show themselves as bizarre grub-like forms. They reproduce by budding.

CHAPTER XVIII.

THE ROUND WORMS.

CLASSIFICATION OF THE NEMATHELMINTHES (ROUND WORMS).

Class.	Family.	Genus.	Species.
Nematoda	Angiostomidæ	Strongyloides	S. stercoralis.
	Filariidæ	Dracunculus	D. medinensis
		Filaria	F. bancrofti
			F. loa
			F. perstans
			F. demarquayi
			F. ozzardi
			F. philippinensis
			F. volvulus
	Trichotrachelidæ	Trichocephalus	T. trichiurus
		Trichinella	T. spiralis
	Strongylidæ	Eustrongylus	E. gigas
		Strongylus	S. apri
		Trichostrongylus	T. instabilis
		Triodontophorus	T. deminutus
		Œsophagostoma	O. brumpti
		Agchylostoma	A. duodenale
		Necator	N. americanus
	Ascaridæ	Ascaris	A. lumbricoides
			A. canis
		Oxyuris	O. vermicularis
Acanthocephala		Gigantorhynchus	G. gigas
Hirudinea		Hirudo	H. medicinalis
		Limnatis	L. nilotica
		Hæmadipsa	H. ceylonica

NOTE.—The Strongyloides stercoralis was formerly described under two designations: (1) Anguillula intestinalis, a parasitic generation and (2) Anguillula stercoralis, a free living generation.

ROUND WORMS OR NEMATODES.

All nematodes are covered by a cuticle which varies in thickness, and is frequently ringed. The cuticle is moulted three or four times. The cuticle is formed by the underlying ectoderm which is, as a rule, markedly developed in four ridges which divide the body into quadrants. Within the ectoderm is the body cavity, a space in which the reproductive organs lie in a clear fluid. The excretory system usually consists of two *tubes which* discharge near the head. The testis and ovary are generally

tube-like. The sexes are, as a rule, separate. The male can usually be recognized by its smaller size, its curved or curled posterior end, and at times exhibiting an umbrella-like expansion—the copulatory bursa. The spicules, chitinous copulatory structures, may be observed drawn up in the worm or projected out of the cloaca. The genital opening of the female is ventral and usually about the mid-point; that of the male is close to the anus.

Certain papillæ in the region of the anus are valuable in differentiation. As a rule nematodes develop in damp earth as rhabditiform larvæ. The families Gnathostomidæ and Anguillulidæ are of very little importance in human parasitology. Gnathostoma siamense was once found in a breast tumor and Rhabditis pellio once in the urine.

ANGIOSTOMIDÆ.

In this family we have heterogenesis.

Strongyloides stercoralis.—This parasite was formerly thought to be the cause of Cochin-China diarrhœa. It presents two generations: 1. Parasitical or intestinal form. 2. The free living or fæcal form.

1. The intestinal form (also known as Anguillula intestinalis) is represented only by females. These are about 1/12 of an inch long and reproduce parthenogenetically. They have a pointed, four-lipped mouth, and a filariform œsophagus which extends along the anterior fourth of the body. The anus is situated near the sharpened posterior end, the vulva about the lower third of the body. They usually live deep in the mucosa and the embryos emerge from the ova laid in the mucosa. The embryos escape from the eggs while still in the intestine, so that in the fæces we only find actively motile embryos. The eggs, which are strung out in a chain, never appear in the fæces except during purgation. As they greatly resemble hookworm eggs, this is a point of great practical importance. In fresh fæces we find hookworm eggs and Strongyloides embryos. The embryos are rather common in stools in the tropics. These embryos have pointed tails and are about 250 x 13μ. They have a double œsophageal bulb. They are about 250μ when they first emerge but may grow until they will approximate 500μ in the fæces. If the temperature is low, these rhabditiform embryos develop into filariform embryos, which being ingested form the infecting stage.

It has been suggested that infection of man may also take place through the skin. If the temperature is warm, 25° to 35° C., these embryos develop into:

2. The free living form, Anguillula stercoralis. In this we have males and females, with double œsophageal bulbs; these copulate and we have produced rhabditiform larvæ, which later change to filariform ones. These, being ingested, start up the parasitical generation. If these do not reach the intestine they die out.

FILARIIDÆ.

This family is of the greatest importance to man. It is also one about which much confusion exists as to the adult type; hence anyone finding adult filariæ should fix them in hot 5% glycerine alcohol (alcohol 70%), and subsequently mount in glycerine gelatin. Formalin is not to be used. These worms are most likely to be seen as writhing thread-like worms, especially in the lymphatic glands and connective tissue, and about body cavities. They have a lipped or simple mouth and a filariform œsophagus. The male has an incurved tail which may be even corkscrew-like as in F. immitis. The spicules are unequal or there may be but one. The female is ovoviviparous and the vulva is at the anterior end.

Filaria medinensis.—The Guinea or Medina worm, of which until recently only the female was known, is of great importance in parts of India, Africa, and Arabia. The female is a thread-like worm, about 20 to 30 inches long. The habitat is the subcutaneous and intermuscular connective tissue, especially of the lower extremity. It develops without symptoms. Finally a blister-like area appears on the surface of the leg, particularly about ankle-joint, which soon forms a painful ulcer. From this opening the anterior end of the worm projects to pour forth its striated embryos upon contact with water. The mouth is terminal and the body uniformly cylindrical. The uterus is a continuous tube filled with sharp-tailed, transversely striated embryos, 650 x 17μ, and constitutes the greater part of the body, the alimentary canal being pressed to one side. The genital organs probably discharge through the œsophagus. The body when being extracted is rather transparent. The tip of the tail is bent, forming a sort of anchoring hook. Recently Leiper fed monkeys on bananas containing infected Cyclops, and at the autopsy *six months* later obtained both male and female forms.

As regards the life history, Fedschenko showed that the embryos when liberated swam around in water and finally entered the bodies of species of the genus Cyclops. The female tends to come to the surface in the lower extremities, and experiments show that if on the blister-like points of emergence some water be squeezed out from a sponge, the uterus will eject a milky-looking fluid containing myriads of embryos.

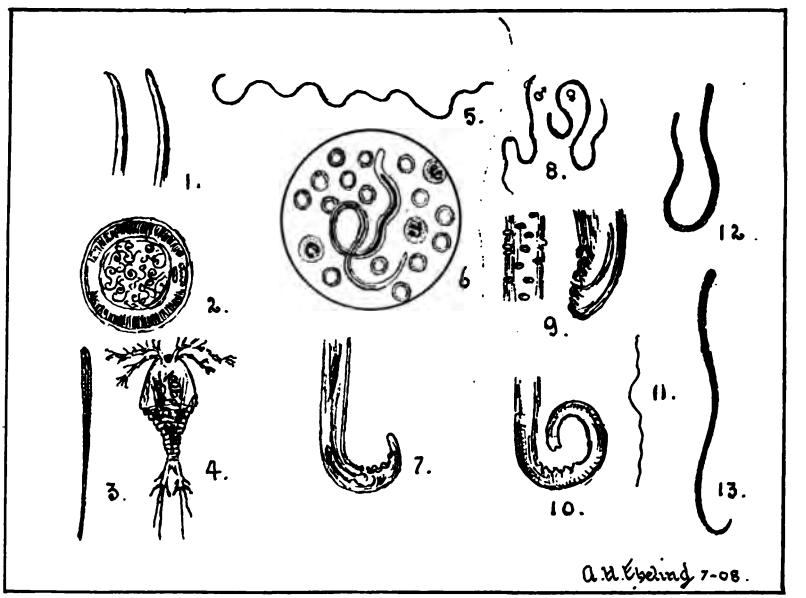


FIG. 64.—Round worms (Filariidæ). 1. Hooked posterior extremity and anterior extremity of Dracunculus medinensis; 2, cross section of uterus filled with embryos, D. medinensis; 3 and 4, free embryo and embryos of D. medinensis in intermediate host (Cyclops); 5, natural size of female Filaria bancrofti; 6, embryo of F. bancrofti in blood; 7, tail of male F. bancrofti; 8, male and female of F. loa (natural size); 9, tuberculated integument and posterior end of male F. loa; 10, posterior end of male F. perstans; 11, male of F. bancrofti (natural size); 12, blunt-tailed embryo of F. perstans; 13, sharp-tailed embryo of F. demarquayi.

This would indicate that the worm selects the lower extremity so that the embryos may gain access to the Cyclops when the host is wading through the water.

Leiper showed that a strength of HCl equal to that of gastric juice killed the Cyclops, but made the Dracunculus embryos very active. From this he judged that infection must probably take place from drink-

ing water containing infected Cyclops. The disease is known as "Dracontiasis."

Filaria loa.—This is a thread-like worm of West Africa about 1 to 2 inches long. The cuticle is characterized by distinct wart-like structures. The anterior extremity is like a truncated cone with two papillæ at the base of the cone.

The males are smaller than the females and have three preanal papillæ and two postanal ones. There are two short unequal spicules. The life history is not satisfactorily established. The young are born ovoviviparously, and it has been suggested that the localized œdemas, known as Calabar swelling, may be due to the irritation produced by these eggs. The embryos almost exactly resemble those of F. bancrofti. They have a diurnal periodicity, however, appearing in the blood about 8 A. M., increasing to noon and disappearing about 9 P. M. The intermediate host is unknown. The adult worms have a tendency to wander about in the subcutaneous connective tissue, especially about the region of the orbit or even under the conjunctiva.

Filaria bancrofti.—This is the most important of the filarial worms. It is a common infection in South China, India, the West Indies, and in the Pacific Islands, especially Samoa.

In medical books the embryos have been designated Filaria sanguinis hominis. This species is the cause of the common manifestations of filariasis, such as elephantiasis, varicose groin glands, chyluria, lymph scrotum, etc.

Filarial diseases are prone to lymphangitis attacks. Thus in lymph scrotum an erysipelatoid condition of the scrotum with high fever and chills may result. This condition is at times mistaken for malaria. Varicose groin glands may be mistaken for hernia.

F. bancrofti lives in lymphatics of trunk and extremities. At times the fine white thread-like worms may be seen as writhing coils excised in lymphatic glands.

The sexes are usually found together. The females are about 3 inches long and the males less than 2 inches. The tails of both sexes are incurved, but that of the male is more so. The head is club-shaped. The sheathed embryos are supposed to be born viviparously and Manson supposes that as a result of injury to the parent worm and resulting extrusion of eggs, the blocking of lymph channels occurs.

A very interesting fact is that people with elephantiasis fail to show

larvæ in the peripheral circulation. Manson considers that it is due to the blocking of the lymph channels.

These embryos show a nocturnal periodicity. During the day they remain in the lungs, and larger arteries.

The disease is transmitted especially by Culex fatigans. The sheathed embryos, getting into stomach of mosquito, wriggle out of the sheath, they then bore their way through walls of stomach and enter into a sort of passive stage, during which further development takes place. They finally become distributed in the muscles of the thorax and make their way along the fleshy labium, to enter the wound in a person bitten by a mosquito, by way of Dutton's membrane. This takes about twenty days at which time the larvæ are about 1/16 inch long and have an alimentary canal.

Filaria perstans.—The adults are found in connective tissue and deeper fat, especially about the mesentery and abdominal aorta.

The female is about 3 inches long; the male is rarely found and is less than 2 inches long. These worms are characterized by incurved tails, the extremity of which has two triangular appendages giving a bifid appearance. The embryos do not possess a sheath and have a blunt tail. The life history is unknown. Both mosquito and tick have been incriminated. The embryos are always present in the peripheral circulation—hence perstans. There does not seem to be any symptomatology.

It is of historical interest that F. perstans was once considered the cause of sleeping sickness.

Filaria volvulus.—This is a rather common parasite of Central Africa. The male is about 1½ inches and the female about 3 inches long. They are striated. They are found in cystic tumors, especially about the axilla and popliteal space. The cystic contents contain abundant sheathless larvæ about 300μ long; they are not found in the peripheral circulation. Life history unknown.

Filaria demarquayi.—The habitat of this filarial worm is the West Indies. The embryo has no sheath and has a sharp tail. Other filarial species which have been reported are F. magalhæsi, F. ozzardi, F. volvulus, F. powelli, and F. philippinensis. A species called F. gigas is now considered to have been only the hair of the leg of a fly. The embryos have usually been given such names as F. nocturna, F. diurna, etc. Of course the embryos and the parent should have the same name. It

has been proposed to designate these embryos the same as the parent, but with the use of the term Microfilaria instead of Filaria.

The points usually noted in the description of filarial embryos are:

1. Presence or absence of periodicity of embryos in peripheral circulation.
2. Presence or absence of a sac sheath around the embryo.
3. Accurate measurements.
4. Shape and description of head and tail ends.
5. Character of movement.

Key to Filarial Larvæ.

A. *Sheath present.*
  1. No periodicity.
    F. philippinensis. Tightly-fitting sheath; not flattened out beyond extremities. Tail is pointed and abruptly attenuated. Lashing progression movement. 320 x 6.5μ.
  2. Periodicity exhibited.
    a. Nocturnal periodicity.
    F. bancrofti (F. nocturna). Pointed tail; loose sheath; lashing movement. 300 x 7.5μ.
    b. Diurnal periodicity.
    F. loa (F. diurna). Pointed tail; loose sheath; cannot be distinguished from F. nocturna except by periodicity. 300 x 7.5μ.

B. *Absence of sheath.* None of these exhibit a periodicity, being continuously present.
  1. Blunt tail—F. perstans. 200 x 4.5μ.
  2. Sharply-pointed tail:
    a. F. demarquayi. 210 x 5μ.
    b. F. ozzardi. 215 x 5μ.

NOTE.—A filarial embryo, F. powelli, reported once. It has a sheath, nocturnal periodicity, and is about 130 x 5μ.

TRICHOTRACHELIDÆ.

These have a long thin neck and a thicker terminal portion. The œsophagus is of the single row of cells type. The anus is terminal; there is only one ovary.

Trichocephalus trichiurus.—This is usually called the whip-worm—the thickened body representing the handle and the narrow neck the lash. It is one of the most common parasites in both temperate and tropical climates. The egg is very characteristic in having an oval shape with knobs at either extremity. It resembles a platter with handles. The male is almost 2 inches long, and has the terminal portion curled up in a spiral. It has a single terminal spicule.

The female is a little longer than the male, and has the terminal part in the shape of a comma instead of being coiled. The neck only contains the œsophagus which is contained in a groove in large cells which form a single row like a string of pearls. These cells play a digestion rôle. The vulva opens at the upper end of the thickened terminal end which contains an intestine lying between the ovary and uterus.

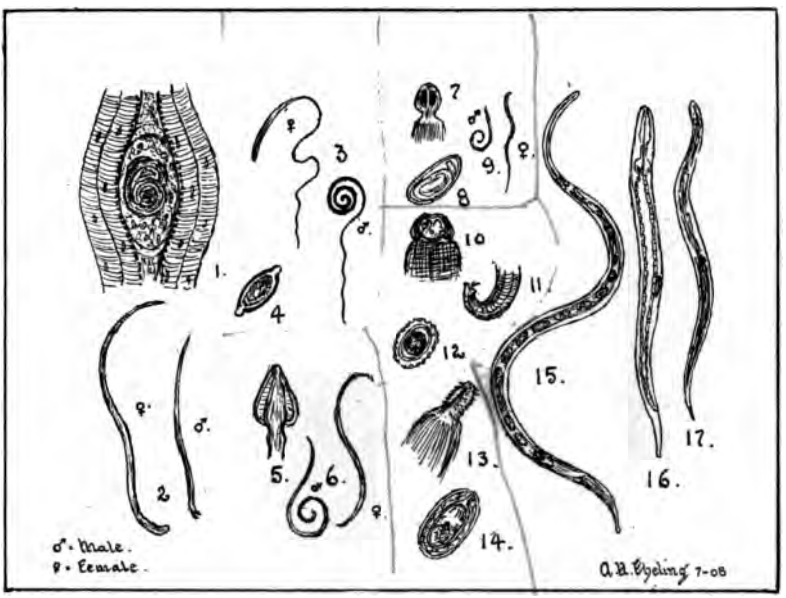


FIG. 65.—Round worms. 1, Encysted embryo of Trichinella spiralis; 2, male and female of T. spiralis; 3, male and female of Trichocephalus trichiurus; 4, egg of T. trichiurus; 5 and 6, head and male and female of Ascaris canis; 7, 8 and 9, head, egg and male and female of Oxyuris vermicularis; 10, 11 and 12, head, egg and tail of Ascaris lumbricoides; 13 and 14, head and egg of Echinorhynchus gigas; 15, 16 and 17, parthenogenetic female and rhabditiform and filariform embryos of Strongyloides stercoralis.

The great powers of resistance of the ova may account for their general distribution; they may live for months under conditions of freezing and so forth. There is no intermediate host. The worm arrives at sexual maturity in about one month after ingestion. The whip-worm prefers the cæcum, but also lives in the lower end of the ileum and the appendix.

The neck burrows into the mucosa, and much importance has been

attributed by the French to the possibility of this paving a way for the entrance of pathogenic bacteria. They do not seem to produce serious symptoms.

Trichinella spiralis.—The cause of trichinosis is usually termed Trichina spiralis in medical works. The adults live in the duodenum and jejunum; the males are about 1/16 of an inch long with two caudal appendages and without a spicule. These two lateral projections enable the male to hold the female in copulation—the cloaca being devaginated.

The females are about 1/6 of an inch long. The female gives off embryos from the vulva which is near the mouth end (viviparous). After fertilization of the females the males die, and the females bore into the intestinal mucosa and begin to produce embryos to the number of

FIG. 66.—Trichina spiralis (*Ziegler*).

more than 1000 each. These gain access to the lymph channels and are distributed by the blood stream to the striated muscles. Embryos reaching other tissues fail to develop.

It is about ten days before they reach the muscle. In the muscle they become encysted as the oval areas containing coiled-up embryos that everyone is familiar with. These oval areas are about 450 x 250μ.

The encysted trichinæ are found chiefly in the muscle fibers of the tongue and diaphragm and may remain alive as long as ten to twenty years; finally, however, the cyst undergoes calcareous infiltration and the embryo dies.

When uncoiled the embryo is about 1 mm. long with the mouth at

the attenuated end. Among cannibals it would be easy to keep the cycle going by eating improperly cooked or raw human meat, the parasite being thus transmitted.

As this would not explain the transmission among civilized men, the following is the life history: Man obtains his infection from eating raw pork, the embryos encysted in the muscle of the hog being liberated in the stomach, and the males and females developing in the intestine as above described. The hog may gain his infection by eating the meat of other hogs or rats. These rats eat scraps of pork at slaughter houses and become infected. Being cannibals, rats when once infected, continue to propagate the infection. In man, while the adults are breeding in the intestine, we have gastrointestinal symptoms. About ten to twenty day after infection the embryos begin to wander and we have the acute muscle pains. In the diagnosis we should try to obtain specimens of the pork which has caused the trouble in order to examine for encysted trichinæ, or to feed to white rats or rabbits, subsequently examining the diaphragm of these animals for encysted trichinæ or the intestine for adult trichinæ. Excision of a small piece of the deltoid may confirm the diagnosis. The best method is to take blood in 3% acetic acid, centrifuge, and examine for larvæ.

During the diarrhœal stage we may examine the stools for adult worms, in particular dead males or possibly actively motile embryos—these latter are about 90 x 6μ.

Always examine the blood for eosinophilia.

It is well to remember that the parts of meat which trichinæ prefer (muscle of diaphragm, of neck, etc.) are often used in sausage.

STRONGYLIDÆ.

In this family the male has a caudal bursa, a prehensile sort of expansion at the posterior end for copulatory purposes.

Eustrongylus gigas.—This is the largest round worm infecting man; it is usually found in the pelvis of the kidney (giant strongyle). There seem to be seven authentic and eight doubtful cases of infection in man.

The females are about 40 inches long and about 1/3 of an inch in breadth. The collar-like copulatory bursa of the male distinguishes it from Ascaris. The source of infection is unknown.

Many of the reported cases were simply fibrinous clots from ureters or wandering round worms.

The very characteristic ova, with gouged-out oval depressions, may be found in the urine, and are diagnostically confirmatory.

Strongylus apri.—This nematode is common in the lungs of hogs, producing a bronchitis in young animals but apparently harmless for adult ones. It has been reported once from the lungs of a six-year-old boy. The male is about 1 inch long with two long spicules. The female is about 2 inches long and has a sharply hooked posterior extremity with the vulva just beyond the bend. The mouth has six lips. The eggs contain embryos when laid.

Trichostrongylus instabilis.—This is a small strongyle formerly known as Strongylus subtilis. The male is about 1/6 of an inch long, and the female about 1/4 of an inch. Anteriorly it tapers to a pointed head end which is only about one-tenth the thickness of the posterior extremity. The male has a bursa and two prominent equal spicules. It has been found in the upper part of the small intestine of inhabitants of Egypt and Japan. It does not appear to produce symptoms. Ova like hookworm ones ($63 \times 41\mu$).

Triodontophorus deminutus.—This is a small round worm with three forked teeth just inside the round mouth opening. They are less than 1/2 inch long and have once been found in the intestinal canal.

Œsophagostoma brumpti.—Six young females were found in a cyst of the colon in an African negro. They were about 1/3 inch long. The anterior end presents an ovoid protuberance. The mouth has six papillæ.

This species has recently been reported by Thomas in a native of Brazil.

Agchylostoma duodenale.—The hookworm, so called from the hook-like appearance of the ribs of the copulatory bursa or from the hook-like projection of the head dorsally, is probably the most important of the parasitic worms. This species in Europe and Africa and the Necator americanus in the New World cause an immense amount of invaliding. The Egyptian anæmia and the Porto Rican anæmia are caused by this parasite. Hookworms may be found in the small intestine (jejunum) of man in enormous numbers. They either produce their effects by feeding on the mucosa or by causing loss of blood. The *males are* little more than 1/3 of an inch long and the females little

more than 1/2 inch in length. The male can readily be distinguished by his umbrella-like expansion or copulatory bursa. The tail of the female is pointed. The vulva of A. duodenale is located in lower half of the ventral surface; that of N. americanus in upper half. The mouth of the Old World hookworm has four claw-like teeth on the ventral side of the buccal cavity and two on the dorsal aspect. In N. americanus

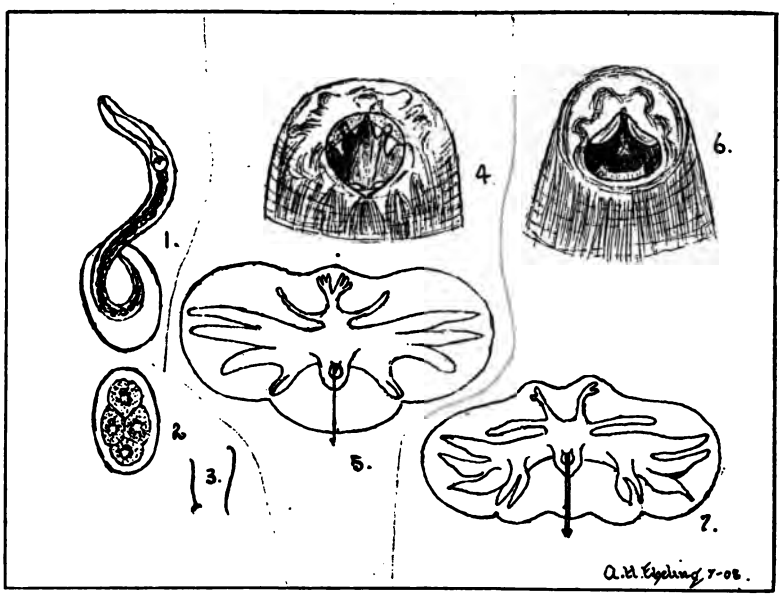


FIG. 67.—Hookworm anatomy. 1, Rhabditiform embryo of Strongyloides stercoralis emerging from egg; 2 and 3, egg and male and female of hookworm; 4 and 5, head and copulatory bursa of male Agchylostoma duodenale; 6 and 7, head and copulatory bursa of male Necator americanus. Dorsal ray, N. americanus shows deep cleavage and bipartite tips.

the buccal capsule is smaller and the ventral teeth are replaced by chitinous plates. The copulatory bursa of the N. americanus is also different, being terminally bipartite and deeply cleft in the division of dorsal ray rather than tripartite and shallow as with the A. duodenale.

The delicate-shelled eggs pass out in the fæces, and in one or two days a rhabditiform embryo (200 x 14μ) is produced. After moulting twice, it remains rather quiescent; it is at this stage that it burrows into the

skin of man, producing the so-called "ground-itch" at the site of entrance. Having gained access to the lymphatics and veins, they eventually reach the lungs. Here they get into the bronchioles and undergo a third moulting. They then work their way up the trachea to the glottis and are swallowed to then become adults in the intestine. Dr. Stiles, while accepting this theory of the life history, thinks it probable that infection is also brought about by swallowing directly some infecting stage.

Very young dogs can be infected with human hookworm larvæ, but infection of man with the dog hook-worm (A. caninum) has not been reported.

The infecting stage is not a young larva but one in which the cuticle of a former larval stage instead of being cast off remains and acts as a protecting sheath for the more mature larva within. In this stage larvæ may remain alive for six to twelve months and have greater powers of resistance than younger larvæ. Introduction, either by skin or mouth, of these cuticle-covered larvæ is followed by finding of eggs in the fæces in about thirty days.

Necator americanus.—This is the species of hookworm found in the southern states of the United States and the West Indies. It is very prevalent in Guam, L. I. It was found by Looss in pigmies from Central Africa, so that this parasite was undoubtedly brought to America by slaves. The copulatory bursa of this parasite has a double spicule one of which is barbed, while A. duodenale has two fine hair-like spicules. The head of Necator has a more marked dorsal bend than Agchylostoma.

To sum up the differences between this species and A. duodenale we have with Necator: 1. Smaller oral cavity which shows rib-like projections leading to the two ventral plates instead of the four prominent projecting teeth. 2. The dorso-median ray of the caudal bursa is deeply cleft and shows bipartite divisions terminally instead of having a shallow cleft and tripartite division. 3. The vulva of the female is placed in the anterior third.

The eggs of N. americanus are larger than those of A. duodenale. In hookworm disease we have ground itch, tibial ulcer, anæmia, interference with physical and mental development and, in bad cases, dirt eating.

ASCARIDÆ.

These have three papillæ around oral cavity, one dorsal and two ventral. The male has two equal-length spicules. An intermediary host is not needed in the life history of this family.

Ascaris lumbricoides.—The male round or eel worm is from 5 to 8 inches long and the female from 7 to 15 inches in length. They are from 1/7 to 1/4 of an inch in diameter.

The normal habitat is the upper part of the small intestine, hence the ease with which they are vomited up. The three papillæ-like lips with a constriction just behind are easily studied with a hand glass. The very long, whitish, convoluted, thread-like tubes of the uterus lead to the opening of the vulva anteriorly and ventrally. The male has two large lance-like spicules.

The body of the worm is transversely striated and resembles the ordinary earth-worm, but is more grayish than red. The ova are very characteristic with a rough mammillated exterior. This at times is shelled off and we have a smooth egg which may be mistaken for eggs of other parasites. The eggs leave the body in the fæces and after a long time—a few weeks to several months, according to temperature—develop an embryo which remains in the shell until swallowed by some man or animal. It is stated that they will remain alive for years. On being swallowed, the embryo leaves the egg and we have males and females developing in the small intestine. In countries where such parasites abound, as in Guam and the Philippines, the possibility of their getting into the peritoneal cavity through operative measures on the intestine must always be thought of.

Guiart considers it probable that ascaris may suck blood, produce intestinal ulcerations and bacterial infections, and perforate intestine. Their entrance into bile ducts or into larynx (vomited) must be considered.

Some think that the symptoms of itching of nose and anus, vertigo, or convulsions and anæmia may be due to a toxin secreted by the worm.

Ascaris canis.—This is a parasite of the dog and cat, but is occasionally found in children. It is much smaller than the A. lumbricoides—male is 2 to 3 inches long, female 4 to 5 inches in length. The parasites are characterized by the presence of wing-like projections from the anterior end (arrow-like head).

Oxyuris vermicularis.—This parasite is also known as the pin-worm or seat-worm. The male is about 1/6 of an inch long and the female a little less than 1/2 inch in length. The male has an incurved tail with a single spicule and the female a long tapering tail. The vulva is in the upper third.

These worms have a clear, slightly bulbous, pipe mouth-piece-like projection surrounding the three-lipped anterior extremity. There is a well-marked bulb œsophagus.

The eggs are thin-shelled, plano-convex, and show a coiled-up embryo. After ingestion of eggs, the adults develop in the small intestine where copulation takes place; the males then die. The fertilized females go to the cæcum and colon where they remain until they reach maturity. At this time the females wander to the rectum where they either expel their ova or themselves work their way out of the anus. This usually occurs at night, and the scratching induced by the itching causes the eggs to be widely spread about the region of the anus. The worms may also wander into the vagina, urethra, or under prepuce. It will be seen that as a result of the scratching, the fingers become contaminated with ova which may be carried to the mouth and so cause a fresh infection A knowledge of the life history—the early location in the small intestine, and later on in the large—shows that treatment should be dual in its direction—enemata for the gravid female in the rectum and santonin and calomel for the young adults in the small intestine.

The diagnosis is preferably made by examining the stools for the white, thread-like females which are expelled after a diagnostic dose of calomel and salts, rather than by searching for the eggs.

ACANTHOCEPHALA.

These are called thorn-headed worms on account of a proboscis which projects anteriorly like a little peg.

There are several rows of hooks surrounding this projection which are directed backward to enable the parasite to attach itself to the intestinal wall. The worm absorbs nourishment through the general body wall, there being no alimentary canal or mouth. These worms are common in hogs. The three-shelled eggs are very striking and the intermediate stage is in June bugs.

***The Echinorhynchus* or Gigantorhynchus gigas.**—This parasite

is about 6 inches long for the male and 10 to 12 inches for the female. It has transverse rings and resembles Ascaris but is more white in color. It is said to be not uncommon in Southern Russia.

The Echinorhynchus or Gigantorhynchus moniliformis might be contracted by persons eating death-watch beetles as is sometimes done for the improvement of the complexion.

HIRUDINEA (LEECHES).

Hirudo medicinalis.—This is the leech used medicinally for the abstraction of blood. They have a secretion which prevents coagulation of the blood so that when they are removed the wound still continues to bleed.

Limnatis nilotica.—This species has been found in many parts of Northern Africa and, gaining access to the stomach through drinking-water, it wanders to the pharynx, nares, and even trachea. Manson refers to a case of obstinate epistaxis and headache caused by a leech in the nostril.

Hæmadipsa ceylonica.—These are land leeches found in India, Philippines, Australia, and South America. They are only about 1 inch long and are slender. They leave the damp earth to climb shrubs and from there to drop on animals or man passing through the forest. The bites are painless, but may be followed by ulcers. They may get into the nostrils.

They will even penetrate thick clothing in order to reach the skin.

CHAPTER XIX.

THE ARACHNOIDS.

Classification of the Arachnoidea.

Order	Family.	Subfamily.	Genus.	Species.
Acarina	Trombidiidæ		Trombidium	T. holosericeum
	Gamasidæ		Dermanyssus	D. gallinæ
	Tyroglyphidæ		Tyroglyphus	T. farinæ T. longior
	Sarcoptidæ		Sarcoptes	S. scabiei
	Demodicidæ		Demodex	D. folliculorum
	Tarsonemidæ		Pediculoides	P. ventricosus
	Ixodidæ	Argasinæ	Argas	A. persicus A. miniatus
			Ornithodoros	O. savignyi.
		Ixodinæ	Ixodes	I. ricinus
			Hyalomma	H. ægyptium
			Rhipicephalus	R. bursa
			Dermacentor	D. reticulatus D. andersoni
			Margaropus	M. annulatus
			Amblyomma	A. hebræum
			Hæmaphysalis	H. leachi
Linguatulida			Linguatula,	L. rhinaria
			Porocephalus	P. constrictus

THE ARACHNOIDEA.

The Arachnoidea differ from the Insecta in having the head and thorax fused together. They also have four pairs of ambulatory appendages, while the insects only have three pairs. The Arachnoidea never have compound eyes—these when present being simple. Of the two orders of Arachnoidea of interest medically the Acarina is far more important than the Linguatulida.

Acarina.

Of the acarines we are chiefly interested in the mites and the ticks. The acarines do not show any separation of the abdomen from the cephalo-thorax. A hexapod larva develops from the egg; this is succeeded by an octopod nymph which differs from the adult in not having sexual organs.

Trombidiidæ.

These generally have a soft, more or less hairy integument and are often brightly colored. The two eyes are often pedunculated and the cheliceræ are styliform and the palps project beyond the rostrum as claw-like appendages. A very common and annoying member of this family is the hexapod larva of the **Trombidium holosericeum.** It is usually designated Leptus autumnalis. Popularly it is termed "harvest mite," "red bug" or "jigger." They are found in the fields in the autumn and attack both man and animals. The condition (itching and redness) produced is at times called autumnal erythema. There is a Trombidium in Mexico which has a predilection for the skin of the eyelids, prepuce, and navel. The Kedani mite is believed by the Japanese authorities to bring about infection with Japanese river fever or Tsutsugamushi, as the result of transmitting either a bacterium or protozoon by its bite. The disease somewhat resembles typhus, although an eschar at the site of the bite and lymphatic involvement is present.

Gamasidæ.

Of the Gamasidæ, which generally have a hard leathery body and styliform piercing cheliceræ, only the Dermanyssus gallinæ is of interest. This coleopterous mite infests chicken-houses and sucks the blood of the inmates. They will also attack man. Poultrymen may be troubled with a sort of eczema on the backs of the hands and forearms, similar to scabies, resulting from bites by these mites. They measure 350 x 650μ. They have no eyes.

Tyroglyphidæ.

Mites of this family live on cheese, flour, dried fruits, etc. They are small, without eyes, and have a smooth skin and a cone-like appearance of the mouth parts. They are chiefly of importance because of their being occasionally found in urine, fæces, etc., and being striking objects, the question of pathogenicity arises. The T. longior has been associated with intestinal trouble (probably a coincidence, patient having eaten cheese containing these mites).

Glyciphagi are found in sugar and are the cause of what is known as "grocers' itch." Rhizoglyphus parasiticus is reported to be the cause of an itch-like affection of the feet of coolies on tea plantations.

To distinguish: the dorsum of Glyciphagus is hairy or plumose; Tyroglyphus has both claws and suckers on tarsi, while Rhizoglyphus has only claws.

Sarcoptidæ.

These are small eyeless mites with a transversely striated cuticle. They live on the epidermis of man and various animals. It is the female that makes the tunnels in the skin between the fingers, on penis,

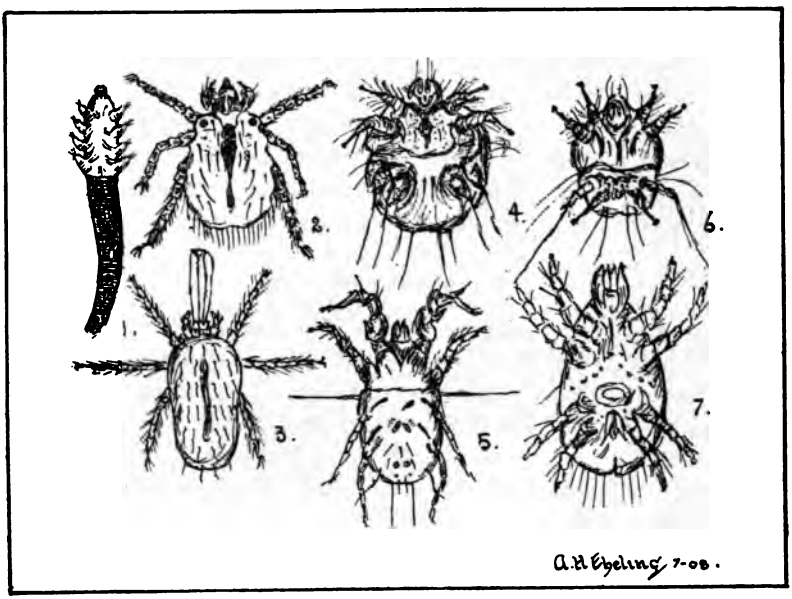


FIG. 68.—Mites. 1, Demodex folliculorum; 2, Kedani mite; 3, Trombidium holosericeum; 4 and 6, Female and male Sarcoptes scabiei; 5, Tyroglyphus farinæ; 7, Rhizoglyphus parasiticus.

flexor surface of forearm, etc. The male dies off after copulation. The female passes through four stages: 1. larva; 2. nymph; resembles adult, but has no sexual organs; 3. the pubescent female; 4. the egg-bearing female. A pair of itch mites may produce 1,500,000 descendants in three months. Transference of eggs, larvæ or pubescent females does not seem to transmit scabies. It is the egg-laden female only. The human itch mite, Sarcoptes scabiei, is an oval mite, the male is 250 x 150μ;

the female is about 400 x 300μ. Besides the difference in size, the male may be distinguished from the female by the fact that the third and fourth pairs of legs in the female have bristles, but in the male, the fourth pair has suckers. The tunnels made by the female have the egg-bearing female at the blind end; scattered all along are fæces, eggs, larvæ; the eggs being next the mother and the more mature young at the entrance to the gallery. A diagnosis can be made from the finding of either eggs or larvæ. The eggs are 140μ long and hatch out in four to five days. A female becomes mature in about three weeks. Different animals have different species of itch mites.

Demodicidæ (Hair Follicle Mites).

Demodex folliculorum.—This is a vermiform acarine about 400μ long; the eggs are about 75μ long; they chiefly live in the sebaceous glands of nose and forehead.

Tarsonemidæ.

This acarine family shows a complete sexual dimorphism. The Pediculoides ventricosus is oval and about 125 x 75μ for the male which has claws at the extremities of the anterior and posterior pairs of legs; the two other pairs have hooklets and a sucking disc. The female is about twice as long but of the same breadth as the male, and has claws only on the anterior legs. The gravid female is like a ball and is about 1000μ in diameter.

They live on wheat and may be found in wheat straw, which, if handled, may be followed by a severe skin eruption with an irregular fever.

Ixodidæ.

This family of the Arachnoidea is one of great medical interest and of growing importance. While only proven the intermediary hosts in the case of the organism of African tick fever and the as yet undiscovered cause of spotted fever of the Rocky Mountains, there is considerable speculation as to the possibility of blackwater fever being due to a Babesia (Piroplasma). Piroplasmata of animals seem to be invariably transmitted by ticks.

Very important diseases due to these small pear-shaped organisms

within red cells are known for various animals, the best known being that of cattle in Texas and known as Texas fever. Other piroplasmata diseases are Rhodesian fever (cattle), heart water (sheep), and malignant jaundice of dogs. In these diseases there are pathological features which resemble blackwater fever of man. It is of interest to note that it was with the transmission of Texas fever through an intermediate host (the tick) that Smith and Kilborne (1889–1893) established the zoological principle of transmission of disease through arthropod intermediary hosts. This led up to the work on malaria, yellow fever, etc. Ticks differ from insects in having four pairs of legs, only two pairs of mouth parts, and no antennæ. They differ from other acarines in having a median probe-shaped puncturing organ, the hypostome, which is beset with numerous teeth projecting backward, and in possessing stigmal plates. The head, or capitulum, or rostrum, is the part which projects anteriorly from the body. This carries the piercing parts which are the single hypostome or dart and a pair of piercing chitinous structures, the cheliceræ. A pair of palpi consisting of segments are on either side of the biting parts. Two depressed pitted areas on the dorsal surface of the capitulum in the adult female are known as porose areas. Very important structures are the stigmal plates. These are striking mosaic-like areas which are located just posterior to each hind leg in the Ixodinæ and between the third and fourth legs in the Argasinæ. As the greatest confusion exists as to the classification of ticks, Dr. Charles W. Stiles has now in hand a system of classifying ticks according to the appearance of these plates as seen under the high power of a microscope. There is great variation in the outline and general picture of these stigmal plates in the different species. The stigmal orifice, the opening of the tracheal system, is in the center. The Ixodinæ have a scutum or shield-like chitinous structure on the dorsal surface. It covers almost the entire back of the tick in the male and only a small portion anteriorly in the female. The genital opening is toward the anterior part of the ventral surface. The anus, with anterior or posterior anal grooves, is near the posterior third of the venter.

Life History of Ticks.—This varies greatly according to the subfamily, genus, and species. The female Ornithodoros savignyi lays about 140 eggs. The larva does not leave the egg, but moults inside, and finally emerges as an eight-legged nymph. It lives in the dust in the cracks of the native huts and comes out at night to feed on the sleeping

natives. As the possibilities for destruction are not so great as with many Ixodinæ the necessity for thousands of eggs is not imperative for the continuation of the species as with the Ixodinæ. With some of the Ixodinæ the females lay from 5000 to 20,000 eggs during several days or weeks and then die. The eggs are preferably deposited near grass. The egg stage lasts from two to six months, when the six-legged larva ("seed tick") emerges. It crawls up a blade of grass and gets on a passing animal. After feeding, or at times without taking nourishment, the larva drops to the ground, and changes to the pupal stage which has four pairs of legs. The pupa crawls up a blade of grass and gets on a passing animal (the second host). Feeding, it falls to the ground where it remains eight to ten weeks. It moults and develops into an adult tick. These males and females gain access to a third animal host—the males fecundate the females, after which the female gorges herself with blood; afterward dropping off the animal and laying eggs. With some ticks fewer hosts suffice.

Classification of Ixodidæ.

Subfamily Argasinæ.—Head concealed by body when viewed dorsally. No scutum. Stigmal plates between third and fourth legs. Adults have no suckers beneath claws. Slight sexual dimorphism. Anus near middle of venter.

Genus Argas.—Body narrow in front. Margins thin and acute. No eyes. The A. persicus (Miana bug) of Persia has been supposed to be concerned in the transmission of a serious disease. Rostrum some distance from anterior margin.

Genus Ornithodoros.—Margins of body rounded. Skin has many irregular tubercles. Rostrum even with anterior margin so that ends of palpi slightly project. O. savignyi has two pairs of eyes near base of mouth parts. It is the intermediate host of Spirochæta duttoni. (South African tick fever.)

Subfamily Ixodinæ.—Mouth parts project in front of body when viewed dorsally. Scutum present. Stigmal plates posterior to fourth pair of legs. Adults have suckers beneath claws.

Anus behind middle of venter.

Sexual dimorphism marked. Male has well-developed scutum; female has porose areas.

CHAPTER XX.

THE INSECTS.

Classification of the Class Insecta.

Order.	Family.	Subfamily.	Genus.	Species.
Rhynchota (Hemiptera)	Pediculidæ		Pediculus	P. capitis
				P. vestimenti
			Phthirius	P. pubis
	Acanthiidæ		Acanthia	A. lectularia
	Reduviidæ		Conorrhinus	C. sanguisuga
Diptera	Pulicidæ	Pulicinæ,	Pulex	P. irritans
			Loemopsylla	L. cheopis
			Ceratophyllus	C. fasciatus
			Ctenocephalus	C. serraticeps
			Ctenopsylla	C. musculi
		Sarcopsyllinæ	Sarcopsylla	S. penetrans
	Simulidæ (buffalo gnats)		Simulium	S. damnosum (mbwa)
	Psychodidæ (sand flies)		Phlebotomus	P. minutus
	Chironomidæ (midges)		Ceratopogon	C. pulicaris
	Culicidæ	Culicinæ,	Culex	C. fatigans
		Anophelinæ	Anopheles	A. maculipennis
	Tabanidæ (horseflies)		Tabanus	T. bovinus
			Hæmatopota	H. pluvialis
			Pangonia	P. beckeri
			Chrysops	C. dispar
	Muscidæ		Glossina	G. palpalis
			Musca	M. domestica
			Auchmeromyia	A. luteola
			Calliphora	C. vomitoria
			Lucilia	L. cæsar
			Chrysomia	C. macellaria (screw-worm)
	Sarcophagidæ		Sarcophaga	S. carnaria
			Ochromyia	O. anthropophaga
	Œstridæ		Dermatobia	D. cyaniventris
			Hypoderma	H. diana

INSECTA.

The class Insecta belongs to the branch Arthropoda. The species belonging to it far outnumber those of any other branch of the animal *kingdom.* Besides the classes Insecta and Arachnoidea, we have the

Crustacea (crabs, lobsters) and the Myriapoda (centipedes, etc.). The two latter are of very little importance medically. The Arthropoda have segmented bodies, but they differ from the worms in having jointed appendages for the purpose of taking in food and moving from place to place. They also have an exoskeleton which is more or less unyielding from the deposit of chitin in the cuticle.

The class Insecta have one pair of antennæ, three pairs of mouth parts (the fused labium being considered as one pair), and three pairs of legs. They have three divisions of the body—head, thorax, and abdomen. The air is supplied by means of tracheæ—branching breathing tubes. Insects have two pairs of wings, the second pair of which is frequently rudimentary and shows simply as knob-like projections. These are termed halteres or balancers. In some insects both pairs of wings are rudimentary, as in the Aphaniptera. Of the class Insecta only the Rhynchota (Hemiptera) and the Diptera are of special importance.

RHYNCHOTA.

The Rhynchota are insects possessing a sucking beak in which the lower lip forms a long thin tube or rostrum which can be bent under the head or thorax. Inside this tube are biting parts—mandibles and maxillæ. The metamorphosis in this order is not marked.

The Pediculidæ.

In this family there are no wings and there is no metamorphosis. The acorn-shaped eggs (nits) are deposited on hairs of the host.

Pediculus capitis.—The female is about 1/12 of an inch long; the male smaller. They vary in color according to the color of the hair of the host. The eggs are deposited on the hairs of the head. The thorax is as broad as the abdomen. There seems to be a marked preference exhibited by lice for their own peculiar racial host. It has recently been suggested that this might account for certain peculiarities in infection where different races were living together and under similar conditions as to food and environment, and yet only one race contracts the disease (beriberi).

Pediculus vestimenti.—This louse lives about the neck and trunk and deposits its eggs in the clothing. It is almost twice the size of the

P. capitis and the abdominal segment is broader than the thorax. It has recently been shown to transmit typhus fever.

Phthirius pubis.—This louse is popularly known as the crab louse. The female is little more than 1/25 of an inch in length, and the male a trifle less. They are almost square. They have a preference for the white race and live about the pubic region. The female lays about a dozen eggs, which hatch out in about a week.

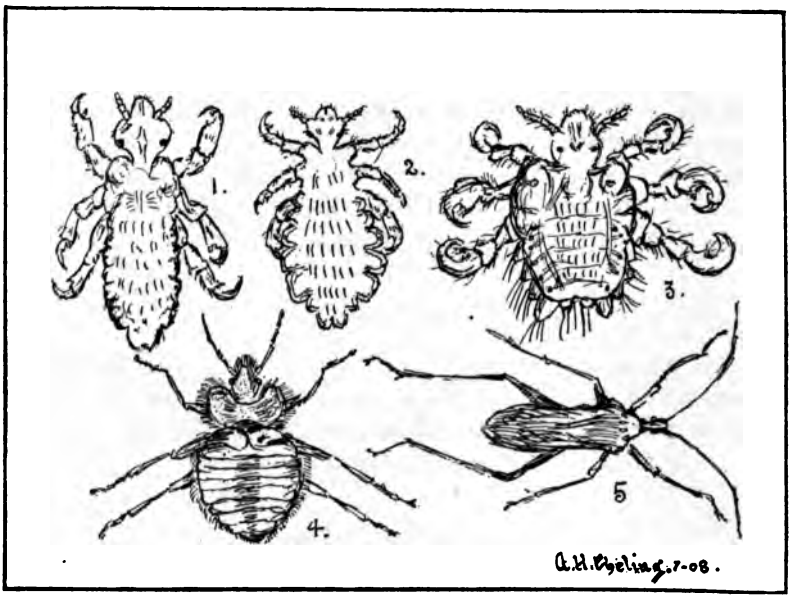


FIG. 70.—Important Rhynchota. 1, Head louse; 2, body louse; 3, crab louse; 4, bed-bug; 5, Mexican bed bug (Conorrhinus sanguisuga).

The Acanthiidæ.

These have a flattened body, a three-jointed rostrum, and four-jointed antennæ. Their wings are atrophied.

Acanthia lectularia.—This is the cosmopolitan bed-bug. It measures about 1/5 by 1/8 of an inch. It is of a brownish-red color. The most conspicuous feature of the bed-bug is the long proboscis continuous with the dorsal integument of the head and tucked under the ventral surface. There are two prominent eyes and two four-jointed

antennæ. There are eight abdominal segments. The bed-bug lives in cracks and crevices, especially about beds. It is said they can migrate from house to house. At any rate, they are frequently transferred with wash clothes. They have a penetrating odor when crushed. The female deposits about fifty eggs at a time in cracks and in ten days they hatch out into larvæ which pass insensibly into adults by a series of five moultings; this depositing of eggs occurs about four times a year.

The bed-bug is very probably the intermediate host in kala-azar and it has been incriminated in connection with typhus fever and relapsing fever.

In India the A. rotundata is the one encountered. It is of a dark mahogany color, has a smaller head and a rather round prothorax. The prothorax of A. lectularia is flattened at the side.

Reduviidæ.

Conorrhinus sanguisuga.—This is known as the Texas or Mexican bed-bug, and was formerly the foe of the common bed-bug, but having gotten a taste for human blood through the Cimex or Ancathia, it now prefers man. It is extending toward the North. It has wings. The bites are much more severe than those of the common bed-bug. It is of a dark brown color, nearly an inch in length, with a long, flat, narrow head and a short thick rostrum. They can run as well as fly. They bite at night.

Diptera.

The insects of the order Diptera are of great importance medically in a variety of ways, either by the direct irritation of their bites, by their transmitting disease directly, as does the common house fly typhoid fever, or by acting as intermediate hosts for various parasites. They are characterized by mouth parts formed for puncturing, sucking, or licking. They present a complete metamorphosis, larva, pupa, and imago. As a rule, the diptera have a distinct pair of wings, the second pair being rudimentary. With the Aphaniptera or Siphonaptera the wings are practically absent. Under the Aphaniptera, we have to consider the Pulicidæ or flea family.

The order Diptera is usually divided into the following suborders: 1. *Siphonaptera:* wingless fleas. 2. *Orthorrhapha:* Diptera with larvæ

having a differentiated head. The imago breaks through the larval or pupal case by a T-shaped break and has no frontal lunule (an oval space just above the root of the antennæ). The Orthorrhapha are divided into: 1. *Nemocera* (with long, many jointed antennæ) and 2. *Brachycera* (with short antennæ). 3. *Cyclorrhapha:* larvæ without differentiated head. The imago escapes through an anterior opening and has a lunule. If the halteres are covered by a scale (squama) we have calyptrate Cyclorrhapha; if not, acalyptrate. 4. *Pupipara:* the larvæ are extruded ready to begin the pupal state.

In studying the biting flies it is very important to recognize the anterior, small, or mid-cross vein. This short transverse rib or vein is the key to wing venation. Beneath it is the discal cell and it bounds the first posterior cell internally or basally. It is also of great value in differentiating Culicidæ. The character of the antennæ should also be noted carefully. The study of the bristles about head, thorax, and abdomen (chætotaxy) is more difficult. Anyone taking up the study of flies should carefully note the wings, etc., of Musca domestica. By putting a few house flies on moist horse manure in a gauze-covered bottle the entire metamorphosis may be observed.

Pulicidæ.

This family is divided into two subfamilies—the Pulicinæ and the Sarcopsyllinæ. In the former the female remains practically unchanged after fecundation, in the latter the abdomen becomes enormously distended with eggs, and the female remains stationary after her impregnation in the burrow which she has made under the skin.

Pulicinæ.—Formerly, with the exception of infection with Dipylidium caninum, the fleas were only under suspicion as carriers of disease; ideas having been entertained as to their being possible transmitters of relapsing fever, typhus fever and kala-azar. As a result of the convincing experiments of the British Plague Commission, their rôle in the transmission of plague has been absolutely established. It is by the bite of the Lœmopsylla cheopis that plague is chiefly transmitted from rat to rat, and in bubonic and septicæmic plague it is apparently the intermediary in human infection. The plague bacilli are passed out with the fæces and these being rubbed into the puncture of the flea bite bring about infection. The puncturing apparatus of the flea consists of a

pointed epipharynx and two mandibles. By the apposition of the mandibles to the epipharynx a tube is formed through which the blood is sucked up. The antennæ are inconspicuous and are in close apposition to the sides of the head, behind the eyes, and can only be well made out with a lens. Fleas have three pairs of legs, and the male can be distinguished from the female by its smaller size and the conspicuous coiled-up penis within the abdomen. The body of the flea is flattened laterally. They may or may not have eyes, and certain conspicuous structures called combs are of importance in classification. In the metamorphosis of the flea the eggs are hatched out in dust of crevices, etc., into bristled larvæ in about one week. The larva forms a cocoon and develops into a nymph which has three pairs of legs. The nymphs emerge from the cocoon as adult fleas in about three weeks after the larva forms it.

Key to the Fleas.

A. With combs.
  1. Eyes present.
    a. Combs along inferior border of head and on prothorax. *Ctenocephalus serraticeps.*
    b. Combs only on prothorax. *Ceratophyllus fasciatus.*
  2. Eyes absent.
    a. Collar of combs on prothorax and four short ones along inferior border of head. *Ctenopsylla musculi.*

B. Without combs.
  a. Ocular bristle arises near upper anterior margin of eye. A line between this and the oral bristle approximately vertical. Two bristles posterior to antennæ. *Lœmopsylla cheopis.* Formerly *Pulex cheopis.*
  b. Ocular bristle arises near lower anterior margin of eye. A line between this and the oral bristle approximately horizontal. One bristle posterior to antennæ. *Pulex irritans.*

The common human flea of Europe is the Pulex irritans; that of the United States the Ctenocephalus serraticeps or dog flea. The flea that is implicated with plague is the Lœmopsylla cheopis. It resembles P. irritans, but is more yellow than brown in color. It also has a greater

number of bristles on the head. The ocular bristle runs above and in front of the eye; that of P. irritans below. It is principally the flea of the mus decumanus, the sewer rat; but the house rat, M. rattus, becomes infected from coming in contact with the sewer rat in the basement.

Sarcopsyllinæ.

Belonging to the subfamily Sarcopsyllinæ, the Sarcopsylla penetrans is of great importance in tropical countries. It is known as the chigoe,

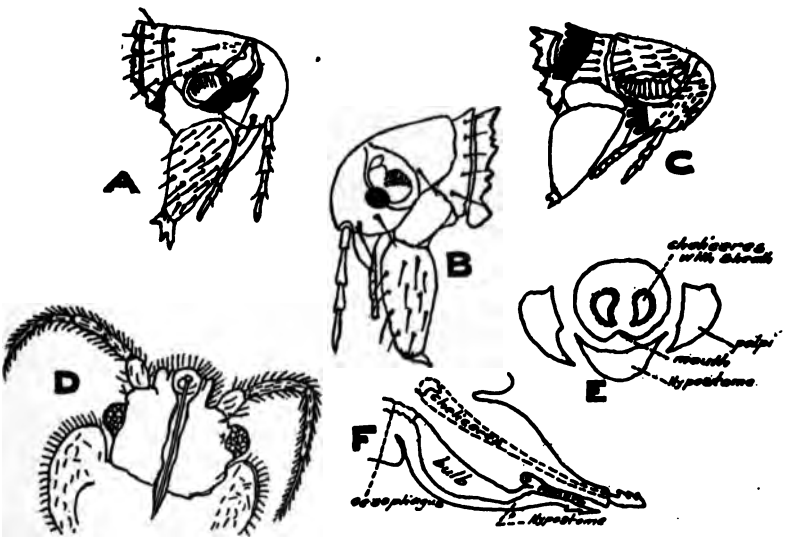


FIG. 71.—Fleas, bed-bugs and ticks. A, Lœmopsylla cheopis; B, P. irritans; C, Ctenopsylla musculi; D, bed-bug; E, cross section of rostrum of Ornithodorus; F. longitudinal section of Ornithodorus.

nigua, or jigger. The male is unimportant. The female, which when unimpregnated is only about 1/24 of an inch long, when impregnated bores its way into the skin of man, especially about the toes, soles of the feet or finger-nails, and in the chosen site develops enormously, becoming as large as a small pea. A small black spot in the center of a tense rather pale area is characteristic. The abdomen of the female is filled with eggs. The metamorphosis is similar to that of the flea. Sarcopsylla can be differentiated from the flea by the proportionately larger head to

the body, and especially by the fact that the head is the shape of the head of a fish, distinctly pointed. With the fleas the lower border of the head comes out in a straight line to join the curve of the upper part. In the Sarcopsylla lower and upper border of head are both curved.

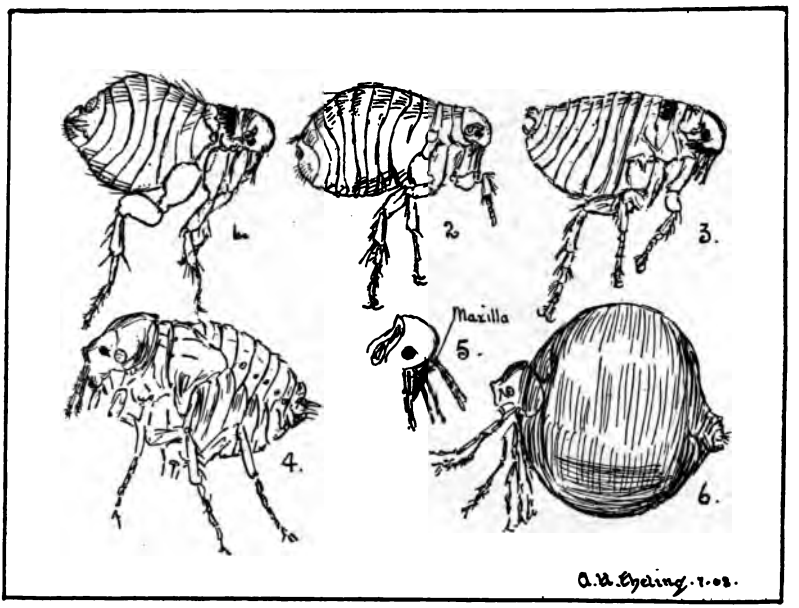


FIG. 72.—Various pulicidæ. 1, Ceratophyllus fasciatus (rat flea); 2, Lœmopsylla cheopis (plague transmitting flea); 3, Ctenocephalus serraticeps (dog flea); 4, Sarcopsylla; penetrans (chigoe); 5, head of flea showing palps; 6, female of S. penetrans distended with eggs after burrowing under skin.

Tabanidæ.

This is the family of horseflies, gadflies, breeze flies or green-headed flies. It is the most numerous family of the Diptera—there being more than 1000 species. The females are blood suckers; the males live on flowers. The eyes are usually very brilliant in color, and in the male make up the greater part of the head.

They belong to the suborder Orthorrhapha and in the group of short antennæ flies (Brachycera). Five posterior cells are always present. No arista. They are thick set flies and rarely show color. The body

of the larva has eleven segments with a small but distinct head. The eggs are deposited in masses on the leaves or stems of plants about marshy places. The larva is carnivorous.

Tabanus autumnalis.—Is about 3/4 of an inch long; it is dark in color, and has four longitudinal bands on the thorax. The last joint of the antennæ has a crescentic notch. The wings do not overlap.

Hæmatopota pluvialis.—In the Hæmatopota there is no crescentic

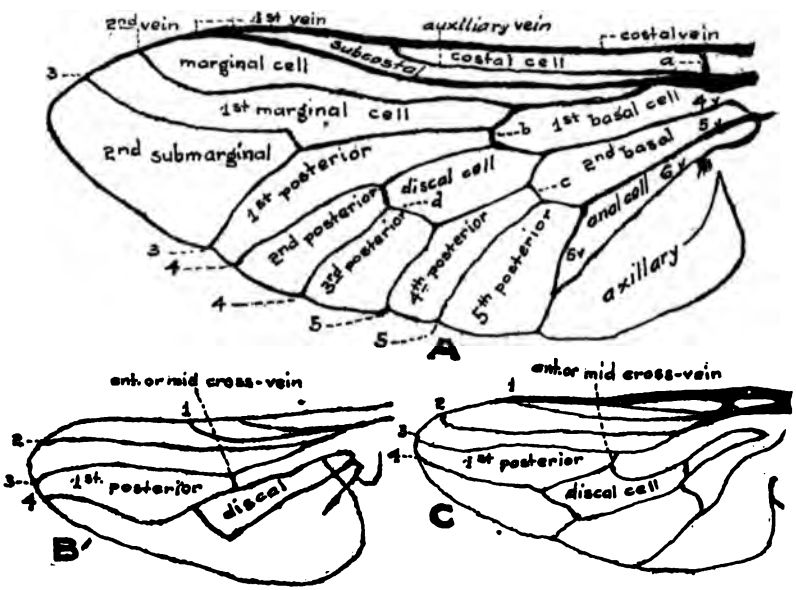


FIG. 73.—Wing venation of A, Tabanus; B, Stomoxys; C, Glossina.

antennal notch, and the wings overlap. The abdomen is narrower than in Tabanus. The brimp, one of the Hæmatopota, bites man severely.

Pangonia beckeri.—The genus Pangonia is characterized by a very long, slender, and more or less horizontal proboscis.

Chrysops dispar.—Chrysops has three ocelli, in this respect differing from the genera Tabanus and Hæmatopota. The wings are widely separated and spotted. The antennæ of Chrysops are especially long and slender. Chrysops and Hæmatopota produce the greatest amount of pain from their bites. The Tabanidæ are not implicated as inter-

mediate hosts in the transmission of disease. By their bites, however, they may transmit disease directly, as with anthrax.

Muscidæ.

The common housefly, **M. domestica,** is the best example of this family. This fly is incapable of biting, but may transmit disease directly, carrying infectious material from the source, as in fæces, to the food about to be ingested. Their rôle in typhoid fever is one of immense importance.

In the Muscidæ the antennæ hang down in front of the head in three segments and have an arista plumose to the tip. The first posterior cell is narrowed. There are no bristles on abdomen except at tip.

FIG. 74.—Common housefly (*Musca domestica*): Puparium at left; adult next, larva and enlarged parts at right. All enlarged. From circular 71 (by L. O. Howard), Bureau of Entomology, U. S. Department of Agriculture.

The Muscidæ, Sarcophagidæ, and Œstridæ are calyptrate Cyclorrhapha.

Stomoxys calcitrans.—These greatly resemble the common housefly in size and shape. They can be easily distinguished by the black, piercing proboscis extending beyond the head. There are longitudinal stripes on the thorax and spots on the abdomen. The proboscis on examination will be seen to be bent at an angle near its base. The palps are short and slender. The wings diverge widely. The genus Stomoxys includes vicious biters. This is the fly which comes into houses before a rain, and which has given the common housefly the reputation of bit-

ing before a rain. Stomoxys may be implicated in transmitting surra (Trypanosoma evansi).

The horsefly (Hæmatobia irritans) rarely bites man. In these the palpi are much longer than in Stomoxys, being as long as proboscis.

Glossina palpalis.—This is the tsetse fly that is responsible for the transmission of human trypanosomiasis (sleeping sickness). The tsetse fly is a small brownish fly about 1/3 of an inch long. The proboscis extends vertically and has a bulb at its base. The arista is plumose

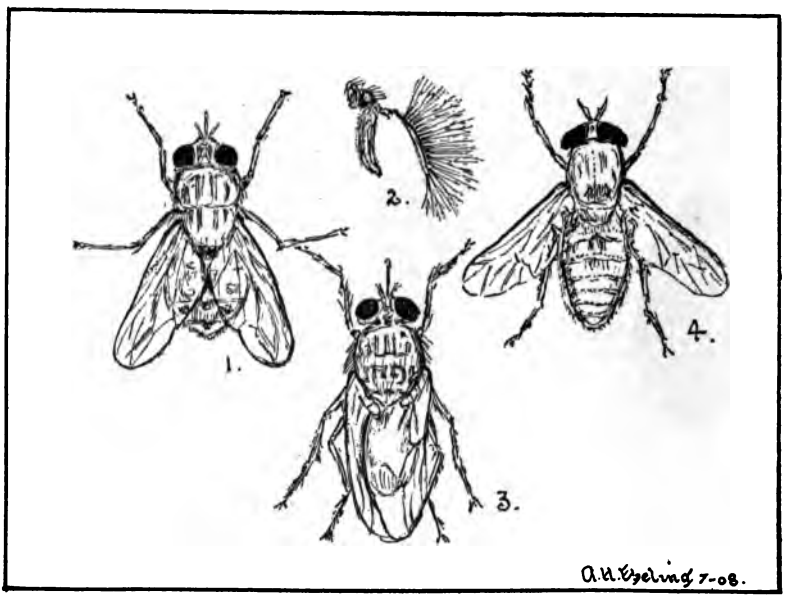


FIG. 75.—Insects in which imago stage is important. 1, Stomoxys calcitrans; 2, arista of Glossina palpalis; 3, Glossina palpalis (tsetse fly); 4, Tabanus autumnalis.

only on the upper side. The wings are carried flat, closed over one another like scissor blades, and project beyond the abdomen. The most characteristic feature of the tsetse fly is the way the fourth longitudinal vein bends up abruptly to meet the mid cross vein and then curves downward to run parallel with the third longitudinal vein. In Stomoxys, the wings separate; in Hæmatopota they just meet, and in Glossina they cross. Glossinæ bite chiefly in the daytime.

The tsetse fly does not lay eggs, but gives birth to a single full-grown

larva which immediately becomes a pupa. Glossina morsitans transmits the cattle trypanosome disease, nagana.

Auchmeromyia luteola.—This is an African fly, the larva of which is known as the "Congo floor maggot," and is a blood sucker. The larva is of a dirty-white color and about 2/3 of an inch long. It crawls out at night and feeds on the sleeping natives. This is the only known instance of a blood-sucking larva.

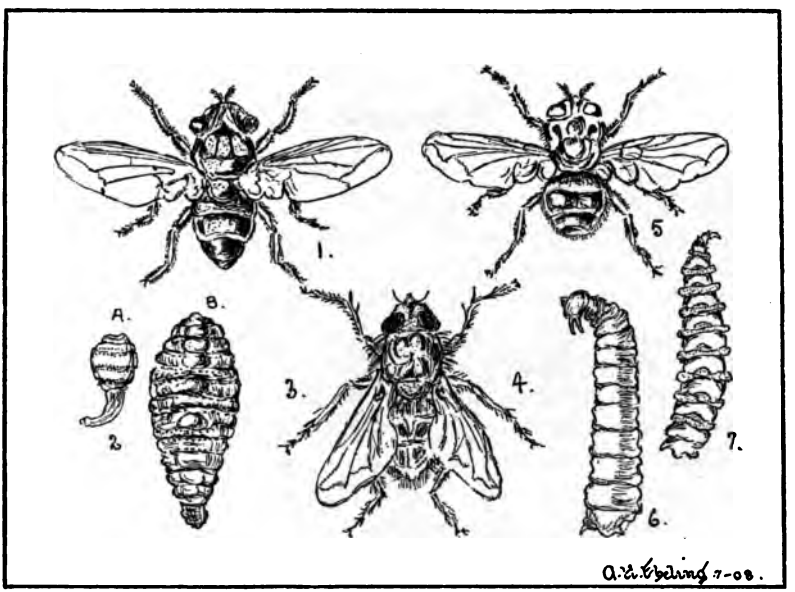


FIG. 76.—Insects in which larval stage is important. 1 and 2, Insect and larval forms of Dermatobia cyaniventris; A, ver macaque, B, Torcel or Berne; 4 and 6, insect and larva of Calliphora vomitoria; 5 and 7, insect and larva of Compsomyia macellaria (screw-worm).

Calliphora vomitoria and Lucilia cæsar.—These are flies with brilliant metallic-colored abdomens, commonly called blow flies in the case of Calliphora and blue-bottle flies for Lucilia. They deposit their eggs on tainted meat and in wounds. Many cases of obscure abdominal trouble are probably due to the larvæ of these flies. Intestinal myiasis is undoubtedly of greater importance than has been thought. The larvæ, with hook-like projections anteriorly and a ringed body, can easily be recognized in the fæces. They have been mistaken for flukes. They

also have a tendency to be attracted by those with ozena and the larvæ may develop in the nostrils.

Chrysomyia macellaria.—This is known as the screw-worm when in the larval stage. The adult fly resembles the blue-bottle flies. It is distinguished from them, however, by the presence of black stripes on thorax. These flies are very common over nearly all North and South America. The thorax is striped. The eggs, which number 250 or more, when deposited in the nostrils or in wounds, develop into the screw-worm larvæ, which may, by going up into the frontal sinus, cause death.

Sarcophagidæ.

These are known as "flesh flies." The most important characteristic is the fact that the arista is plumose up to the mid-point, beyond which it is bare. They are usually thick set.

Sarcophaga carnaria.—This is a grayish fly with three stripes on thorax and black spots on each segment of the abdomen. It is viviparous. The larvæ gain access to nasal and other cavities and there develop. Cases of death have been reported. Naturally, the fly deposits its larvæ on decaying flesh. In times of war all of these flies become important by reason of "maggots" in the wound. These larvæ are the most common ones in intestinal myiases. The mouth hooklets are strongly curved and separate. Each abdominal segment has a girde of spines. The anterior end is somewhat pointed. The hind stigmal plate is in a deep cavity.

Ochromyia anthropophaga.—This is an African fly whose larvæ develop under the skin of man and animals. It is known as the Ver de Cayor. The larva resembles the Ver Macaque.

Œstridæ.

The flies of this family are usually called botflies. The mouth parts are almost vestigial. They have a large head with a somewhat bloated-looking lower portion. They are often rather hairy.

Dermatobia cyaniventris.—These are large, thick-set flies about 3/5 inch long, with prominent head and eyes, small antennæ, and a marked narrowing at the junction of thorax and abdomen. The

thorax is grayish and the abdomen a metallic blue. The larvæ are deposited under the skin in various parts of the body. When the larvæ move they cause considerable pain. At first the larva is club-shaped, but later on it becomes oval. The former is called Ver Macaque, the latter Torcel.

Hypoderma diana.—The larval form of this fly has been reported three times for man.

In Hypoderma the arista is bare while in Dermatobia the upper border is plumose.

CHAPTER XXI.

THE MOSQUITOES.

MOSQUITOES (Culicidæ) are of the greatest importance medically, not only from their influence upon health in general by reason of interference with sleep and possibly from direct transmission of disease, but, more specifically, they are the only means by which it at present appears possible to bring about infection with such diseases as yellow fever, malaria, filariasis, and possibly dengue. In addition, many diseases of animals are transmitted by mosquitoes.

The Culicidæ differ from all other Diptera in having scales on their wings and generally on head, thorax, or abdomen.

To identify a mosquito, examine a wing and note the scales; also note the presence of two distinct fork cells and, in addition, that the costal vein passes completely around the border of the wing, making a sort of fringe with its scales. Mosquitoes undergo a complete metamorphosis, there developing from the egg a voracious, rapidly-growing larva; next, a nongrowing, nonfeeding stage—the pupa or nymph. There the head and thorax are combined in an oval body, from the back of which projects the siphon tubes; and tucked in ventrally is a small tail-like appendage.

The fully developed insect emerges from the pupa.

The Culicidæ belong to the suborder Nematocera. These have long articulated antennæ and include four families: Culicidæ, Chironomidæ, Simulidæ, and Psychodidæ.

The principal mosquito-like, blood-sucking Diptera which are frequently mistaken for mosquitoes—none of which have scales on their wings—are the following:

1. **Chironomidæ** or Midges.—The blood-sucking species of Chironomidæ, which are found in most parts of the world, belong chiefly to the genus "Ceratopogon." These midges are of very small size, about 1/12 of an inch long, are able to get through netting and, usually being in swarms, they

are exceedingly troublesome. The antennæ have thirteen joints and the wings are shorter than the abdomen and have only longitudinal veins. One of the midges, the "jejen" of Cuba, is a great scourge, its small size enabling it to enter eyes and nostrils. The larva of Chironomus is a red worm-like creature; the pupa has a tufted head.

2. **Simulidæ** or Buffalo Gnats.—These are small blood-thirsty insects only about 1/8 of an inch in length. The thorax is humped, the legs are short and the proboscis short and inconspicuous. The antennæ have eleven joints. One species, the S. damnosum, known by the natives of Uganda as "Mbwa," is greatly dreaded; its bites causing swellings and sores.
3. **Psychodidæ.**—These are small, hairy, slender midges, with long legs and a long proboscis. They are only about 1/12 of an inch in length. The wings have numerous longitudinal veins. Some, as Phlebotomus, are vicious biters. It has been suggested that they may be of importance in the transmission of tropical ulcer. The proboscis is much shorter than that of mosquitoes.

Mosquitoes have three main parts of the body—the head, the thorax, and the abdomen. On the head, the space behind the two compound eyes is called the frons, in front, and the occiput posteriorly.

The nape is back of the occiput. The bulbous prolongation of the frons which projects over the attachment of the proboscis is the clypeus. The clypeus is hairy in the Culex; scaly in Stegomyia. The proboscis is straight in all mosquitoes of importance medically. It consists of a fleshy, scaled, gutter-shaped portion beneath, known as the labium, which terminates in two hinge-joint processes—the labella. At the end of the labium is a thin membrane (Dutton's membrane). It is through this that filarial embryos are supposed to pass on their way from the interior of the labium to enter the person bitten. The labium may be considered as the sheath of a knife, holding and protecting the slender, blade-like penetrating organs. Lying in this groove we have, from above downward, the horseshoe-shaped labium—epypharynx, the under surface of which is open. This when closed by the underlying hypopharynx forms a tube through which the blood is sucked up

by the mosquito. In the hypopharynx, which somewhat resembles a hypodermic needle, is a channel, the veneno-salivary duct. It is down this channel that the malarial sporozoite passes. There are two pairs of mandibles and two pairs of maxillæ on either side of the hypopharynx—the mandibles above and the maxillæ below. The serrations of the maxillæ are coarser than those of the mandibles. The sensory organs,

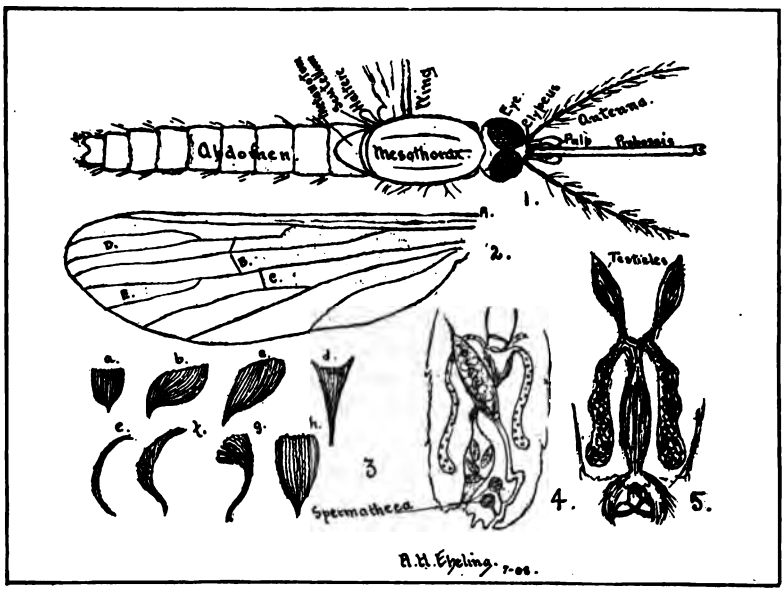


FIG. 77.—Anatomy of mosquito. 1, Dorsal view of mosquito; 2, wing of mosquito: A, costal vein; B, mid cross vein; C, posterior cross vein; D, first fork-cell; E, second fork-cell; 3, various types of scales; a, flat head scales; b and c, Mansonia wing scales; d, upright forked head scales; e, f, g and h, various shapes of thoracic scales.

the palps, lie on either side of and slightly above the proboscis. These are of the utmost importance in differentiating mosquitoes and must not be confused with the antennæ, which are attached above the palpi and at the sides of the clypeus. These antennæ are of importance in distinguishing the sex of the mosquito.

The thorax is largely made up of the mesothorax, at the posterior margin of which is a small, sharply-defined piece, the scutellum; this may be smooth or trilobed. Underneath and posterior to the scutellum

is the metanotum; the metanotum is bare in Culicinæ, has hairs in Dendromyinæ and scales in Joblotinæ.

There is a pair of wings attached to the posterior part of the mesothorax and, more posteriorly still, a pair of rudimentary wings (halteres) attached to the metanotum. The three pairs of legs are attached to the thorax.

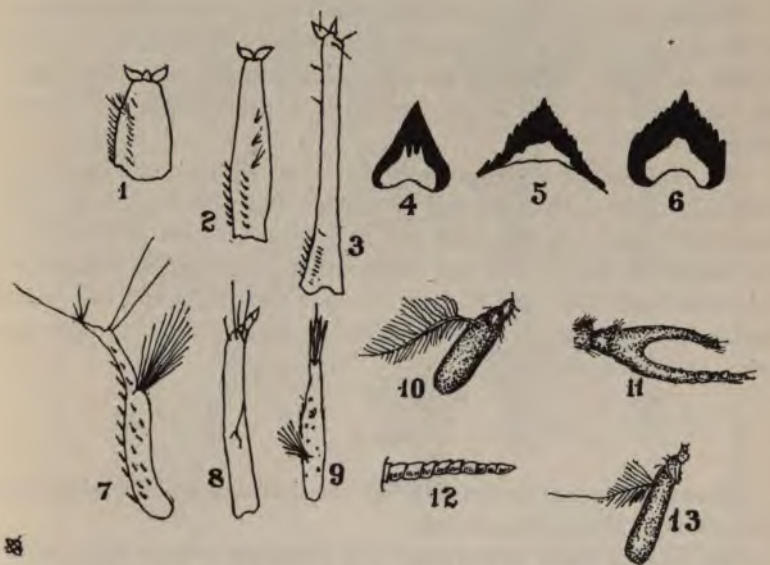


FIG. 78.—Distinguishing characteristics of mosquito larvæ and fly antennæ. Siphon tubes of 1, stegomyia, 2, culex, 3, tæniorhynchus; mental plates of 4, Tæniorhynchus, 5, Stegomyia, 6, Culex;, larval antennæ of 7, Culex, 8, Stegomyia, 9, Anopheles; antennæ of 10, Muscidæ, 11, Tabanidæ, 12, Similidæ, 13, Sarcophagidæ.

There are nine segments in the abdomen. The genitalia arise from the terminal segments as bilobed processes. In the male there is a pair of hook-like appendages or claspers, between which, and ventrally situated, are the harpes, also a pair of chitinous processes.

In considering the question of the possible danger which might arise from the introduction of a case of yellow fever, malaria, or filariasis, it would give the greatest information if mosquito ova were at hand so that we could by watching the development from egg to larva, pupa, and insect, have all the points from which to decide as to the genera developing in

the given locality. It is generally a very easy matter to dip out large numbers of larvæ from the pools and having noted the characteristics of the larvæ, to do the same when the pupæ develop; so that we have only to verify our identification when the insect emerges from the pupa.

THE OVA.

The egg raft of Culex, containing about 250 ova, is quite perceptible on the surface of the water as a black, scooped-out mass, about 1/5 of an inch in length. The eggs are set vertically in the raft. The eggs of the Stegomyia are laid singly and have a pearl-necklace-like fringe around them.

The Anophelinæ eggs are oval in shape with air-cell projections from either side. They are laid in triangle and ribbon patterns. The markings of these air cells vary and have been used for differentiation. The length of time of the egg stage varies according to temperature and other conditions—one to three days for Stegomyia and two to four days for Anophelinæ. The Anophelinæ are more difficult to raise than Culex or Stegomyia.

LARVÆ.

There are two great classes of larvæ—the siphonate and the asiphonate. The latter are always Anophelinæ.

The Culicinæ larvæ have a projecting breathing tube at the posterior extremity which is called a respiratory siphon. This projects off at an angle from the axis of the body, the true end of which terminates in four flap-like paddles. If you divide the length of the siphon by the breadth, you get what is known as the siphon index. In Culex the siphon is long and slender; in Stegomyia it is short and barrel-shaped. When at the surface the Culex larva has its siphon almost vertical and the body at an angle of about 45°.

The Stegomyia larva hangs more vertically. As a rule, the hairs proceeding from the sides of Culex larvæ are straight and the head relatively large. There are also no palmate hairs along the sides.

The Anophelinæ larvæ have a small head which is capable of being twisted around with lightning-like rapidity. They are darker in color *and have no siphon*; float parallel to the surface of the water; have long

lateral branching hairs, and on the sides of each of the five or six middle abdominal segments they have a pair of palmate hairs. These palmate hairs are supposed to aid them in keeping their position on the surface of the water. The larvæ are usually called "wigglers." The duration of the larval stage is from 1 to 2 weeks, according to the temperature.

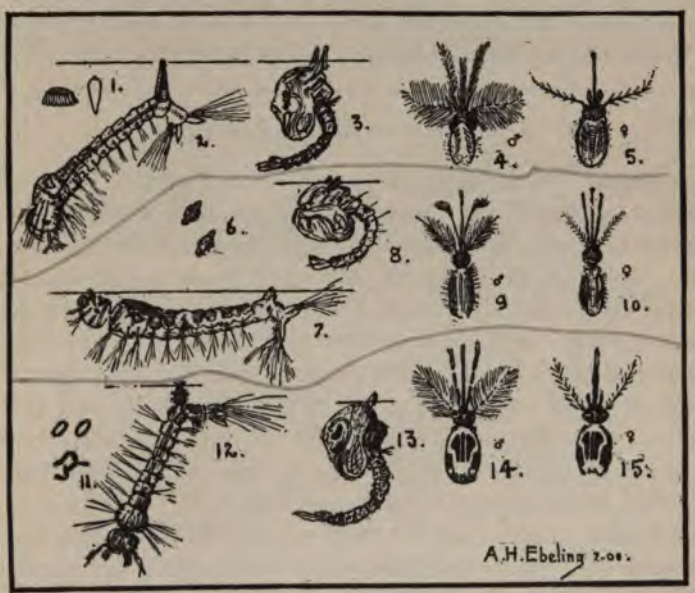


FIG. 79.—Metamorphosis of mosquitoes. 1, 2, 3, 4, and 5, Eggs, larva, pupa, and heads of male and female Culex; 6, 7, 8, 9, and 10, eggs, larva, pupa, and heads of male and female Anopheles; 11, 12, 13, 14, and 15, eggs, larva, pupa, and heads of male and female Stegomyia.

THE PUPÆ.

These have a bloated-looking cephalo-thorax and a shrimp-like tail—the latter the abdomen. Very important in examining them with a lens is to note the characteristics of the siphon tubes which project from the dorsal surface. These siphons are long and slender in Culex and project from the posterior portion of the head end. In Anophelinæ they are broadly funnel-shaped and arise from the middle of the head end. The siphon of the Stegomyia is triangular.

The bulbous end of the Culex nymph is more vertical than the

horizontally-placed cephalo-thorax of Anopheles. The duration of pupal life is short—only one to three days. At the end of this time the pupa comes to the surface and straightens out. The integument then splits dorsally and the perfect insect emerges. It dries its wings for a time on its raft-like pupal skin and then flies away.

From the above it will be seen that the stages in the metamorphosis of the mosquito take about two weeks: one to three days for egg stage; seven to ten days for larval stage, and two to three days for pupal stage.

Dissection of the Mosquito.

The easiest way to secure a mosquito for dissection is to use an ordinary plugged test-tube. Slipping the open end of the test-tube over the resting mosquito, by a slight movement, the insect will fly toward the bottom. Then quickly insert the plug. If it is not desired to study the scales, the best way to kill the mosquito is by striking the tube sharply against the thigh. If it is also desired to study the scale characteristics it is better to put a drop or so of chloroform on the lower part of the cotton plug. The vapor falls to the bottom of the tube and kills the mosquito. Take the mosquito out, pull off legs and wings, and then place the body in a drop of salt solution on a slide. Then hold the anterior end of the thorax by pressure of a needle. With a needle in the other hand, gently crush the chitinous connection between the sixth and seventh segments of the abdomen. Then holding the thorax firm, steadily and gently pull the last segments in the opposite direction. If this is done properly, a delicate gelatinous white mass will slowly float out in the salt solution. One should be able to secure the alimentary canal as far up as the proventriculus, which is just anterior to the stomach, the part in which the malarial zygotes develop. Proceeding from before backward, we have the proventriculus, which is a sort of muscular ring at the opening of the stomach or mid-gut. Leading from the stomach we have the hind-gut, which ends in the rectum. Taking origin at the posterior end of the stomach and festooning the hind-gut are five longitudinal tubes—the Malpighian tubules. These are characterized by large granular-like cells with a prominent refractile nucleus. They are regarded as the renal structures. It is in these tubules that the embryo of the Filaria immitis of the dog develops. In the female mosquito, the parts withdrawn may seem to be largely made up of the white oval

ovaries. These are connected with the spermathecæ, in which the spermatozoa are stored after fecundation by the male. In the male the testicles are quite distinct. Next to the examination of the stomach for zygotes, which appear as wart-like excrescences on its outer surface, the most important structures are the salivary glands, where the malarial sporozoites are found. The easiest way to dissect out the salivary glands is to press down firmly, but gently, an the anterior part of the thorax, and then with the shaft of a second needle, pressing on the head, to gently

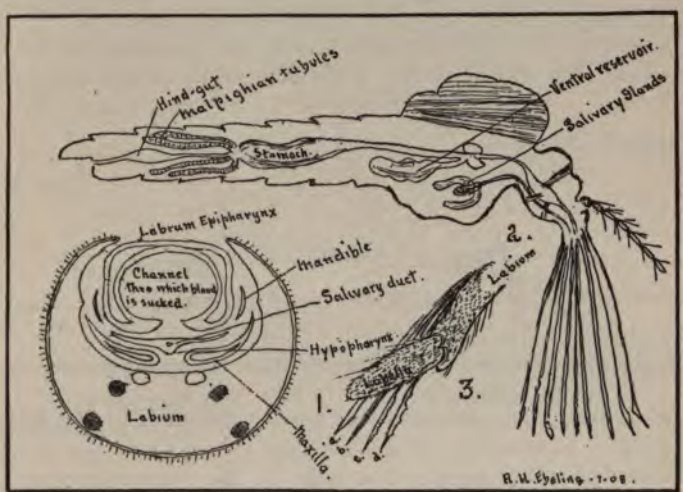


FIG. 80.—Anatomy of mosquito. 1, Cross section of proboscis of mosquito; 2, anatomy of mosquito, longitudinal section; 3, tip of proboscis of mosquito; a, labrum-epipharynx; b, hypopharynx; c, mandible; d, maxilla.

draw the head away from the thorax, so that by this expression and traction movement you extract them with the head segment. They are very minute and are to be told by their exceedingly high refractile appearance. To stain for sporozoites, pick up the head end, and with forceps draw the severed neck along a clean dry slide, trying at the same time to smear out the adherent salivary glands. After drying, stain with Wright's stain. The sporozoites are narrow falciform bodies about 12μ in length, with a central chromatin dot.

A matter about which there is dispute is as to whether the salivary glands communicate with the alimentary canal. Theobald states that there is no connection between them.

Differentiation of Culicinæ and Anophelinæ.

It is impossible even for an entomologist to differentiate mosquitoes without recourse to elaborate keys and tables. It is a comparatively easy matter, however, to decide as to whether the mosquito is a probable malaria transmitter or not.

While certain characteristics of the male are used to separate the Ædinæ from other subfamilies, yet it is only with the female that we concern ourselves in differentiating the Culicinæ from the Anophelinæ. Therefore, it is first necessary to distinguish the male from the female. If the antennæ have not been torn off, this can be decided by the highly-adorned plumose antennæ of the male, those of the female being sparsely

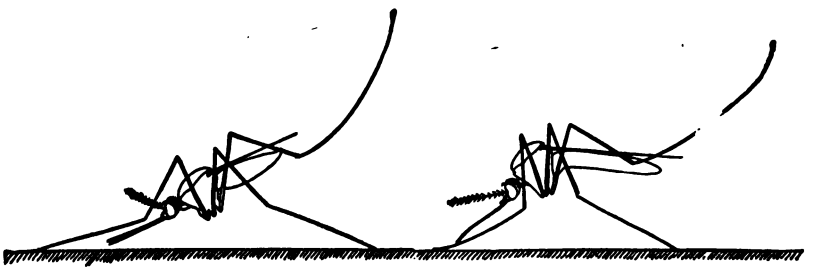


Fig. 81.—Anopheles. Fig. 82.—Culex.
Resting positions of anopheles and culex insects. (*Drawn by C. O. Waterhouse.*)

decorated with short hairs. The palpi of the Anophelinæ tend to be clubbed, while those of the Culex are straight. If the antennæ have been broken off, look for the claspers at the end of the abdomen. Having determined that the insect is a female, we then proceed to place it either in the subfamily Culicinæ or Anophelinæ by a study of the relative length of the palpi to the proboscis. If the palpi are shorter than the proboscis, it belongs to the Culicinæ; if as long or longer, to the Anophelinæ. The palpi of the female Megarhininæ are also long, but the proboscis is curved.

Having settled on the subfamily, we separate the genera by considering such points as character and distribution of scales on back of head, wings, thorax, and abdomen; banding of proboscis, legs, abdomen, and thorax, shape of scales on wings, and location of cross-veins.

In the resting position Culex allows the abdomen to droop, so that it is parallel to the wall. The angle formed by the abdomen with head and proboscis gives a hunchback appearance.

Anopheles when resting on a wall goes out in a straight line at an angle of about 45°. It resembles a bradawl.

Classification.

There are four subfamilies of Culicidæ, differentiated according to the palpi:

1. Palpi as long or longer than proboscis in male.	1. Palpi as long as proboscis in females; proboscis straight. *Anophelinæ.* 2. Palpi as long or shorter than proboscis; proboscis curved. *Megarrhininæ.* 3. Palpi shorter than proboscis. *Culicinæ.*
2. Palpi shorter than proboscis in male and female. *Ædinæ.*	

The important ones from a medical stand-point are the Anophelinæ and Culicinæ.

Anophelinæ.

1. Scales on head only; hairs on thorax and abdomen.	1. Scales on wings, large and lanceolate. *Anopheles.* Palpi only slightly scaled. 2. Wing scales small and narrow and lanceolate. *Myzomyia.* Only a few scales on palpi. 3. Large inflated wing scales. *Cycloleppteron.*
2. Scales on head and thorax (narrow curved scales). Abdomen with hairs.	1. Wing scales small and lanceolate. *Pyretophorus.*
3. Scales on head and thorax and abdomen. Palpi covered with thick scales.	1. Abdominal scales only on ventral surface. Thoracic scales like hairs. *Myzorhynchus.* Palpi rather heavily scaled.
	2. Abdominal scales narrow, curved or spindle-shaped. Abdominal scales as tufts and dorsal patches. *Nyssorhynchus.*
	3. Abdomen almost completely covered with scales and also having lateral tufts. *Cellia.*
	4. Abdomen completely scaled. *Aldrichia.*

NOTE.—Of the above genera only Cycloleppteron and Aldrichia are unproven malarial transmiters.

The Megarhininæ are of no importance medically.

The genus Megarhinus has the following characteristics:

1. Large mosquitoes with brilliant metallic coloring. (Elephant mosquitoes.)
2. Long, curved proboscis.
3. Caudal tufts of hairs on each side of abdomen.

The Ædinæ are not known to play any rôle in transmission of diseases. This subfamily is characterized by having the maxillary palpi much shorter in both males and females than the proboscis.

One genus Sabethes is very characteristic, owing to dense paddle-like scale structures on two or more legs.

Differentiation of Culicinæ Genera.

1. Posterior cross-vein nearer the base of the wing than the midcross-vein.
   1. Proboscis curved in female. *Psorophora.*
   2. Proboscis straight in female.
      A. Palps with three segments in the female.
         a. Third segment somewhat longer than the first two. *Culex.*
         b. The three segments equal in length. *Stegomyia.*
      B. Palps with four segments in the female.
         a. Palps shorter than the third of the proboscis. Spotted wings. *Theobaldia.*
         b. Palps longer than the third of the proboscis. Irregular scales on wings. *Mansonia.*
      C. Palps with five segments in the female. *Tæniorhynchus.*
2. Posterior cross-vein in line with midcross-vein. *Joblotina.*
3. Posterior cross-vein further from base of wing than midcross-vein. *Mucidus.*

Of the Culicinæ the genus Stegomyia is of importance on account of yellow fever. The totally efficient hosts for filariasis (filarial embryos found in the thorax and proboscis) are chiefly among the genus Culex. The genera Mansonia and Tæniorhynchus may also transmit filariasis. Some think the Anophelinæ genera "Cellia" and "Myzomyia" may transmit filariasis as well as malaria.

The genus Culex is implicated in dengue.

Stegomyia.—This is the most important culicine genus. These are mosquitoes with silver markings. The head, entirely covered with flat scales, has also some upright forked scales. Scutellum has dense flat scales. *S. calopus* is deep blackish-brown with two thoracic parallel

lines with curved silver-white lines outside (lyre marking). Banding of thorax, abdomen, and legs.

S. scutellaris has a single silver stripe down the center of thorax. Mosquitoes of this genus are often called "Tiger mosquitoes." The larvæ have short, barrel-shaped siphons. They breed particularly in receptacles about the house.

Culex.—Male palpi long and acuminate. Head has narrow curved and upright forked scales. Laterally, flat scales. C. fatigans supposed to carry dengue.

Theobaldia.—These Culicinæ have spotted wings resembling Anophelinæ. These spots are due to aggregations of scales, not to dark scales. Male palps are clubbed (like Anopheles).

Mucidus.—This genus has a mouldy look from long twisted gray scales. The legs are densely scaled.

Mansonia.—This genus is characterized by broad flat asymmetrical wing scales.

Grabhamia.—Wings have pepper-and-salt appearance with short fork cells.

Tœniorhynchus.—This genus is characterized by dense wing scales, which are broadly elongated with truncated apex.

Acartomyia.—Much like Grabhamia, but scales of head give ragged appearance. Male palpi clubbed.

A. zammittii was supposed to be concerned in Malta fever, but it is now known that transmission is by medium of milk of infected goats.

CHAPTER XXII.

POISONOUS SNAKES.

SNAKES belong to the class Reptilia and the order Ophidia. They are divided into colubrine snakes (Colubridæ) and viperine snakes (Viperidæ).

Of the Colubridæ the Hydrophinæ or sea-snakes with rudder-like compressed tail and the Elapinæ with round tails are most important.

The cobras belong to the subfamily Elapinæ and are best known by a neck-like expansion or hood. The only poisonous colubrine snakes in the United States are the beadsnake (Elaps fulvius) often called the Florida coral snake, and the sonoran coral (Elaps euryxanthus). Although small, they are very venomous. The upper jaw has fangs, which appendages are not present in the nonpoisonous coral snakes, these latter having teeth in the upper jaw so that the wound shows four rows of punctures instead of two rows and one larger puncture on each side to mark the entrance of the fangs.

In Asia there are many important poisonous colubrine snakes; the cobra (Naja tripudians), the King cobra (Naja bungarus) and the Kraits (Bungarus fasciatus).

All of the Australian poisonous snakes are colubrines.

The Viperidæ are the most important poisonous snakes in America. The rattlesnake (Crotalus), the copperhead (Agkistrodon), and the water moccasin being widely distributed in the United States.

There are many harmless snakes which more or less resemble these "Pit Vipers" as the rattlers, moccasins, and copperheads are called. This term refers to a deep hole or pit found on the side of the head between the nostril and the eye. It is a blind sac.

The poison fangs are grooved or perforated and connected with the poison glands which resemble salivary glands and may be almost an inch in length in large snakes. The tongue is slender and forked and is a tactile organ.

In addition to the possession of the pit, these vipers have a more or less triangular head and in particular a single row of large scales on the *under* surface posterior to the vent (anus), while the harmless snakes

show an elongated oval head and two rows of large ventral scales posterior to the vent.

In examining the wound made by a snake the two punctures of the fangs indicate the bite of a poisonous snake. If these fang puncture points are far apart it shows that a large snake, and probably one capable of injecting a greater amount of venom has given the bite.

When a snake strikes the fangs move from the horizontal to the erect position, the mouth being widely open. When the fangs enter

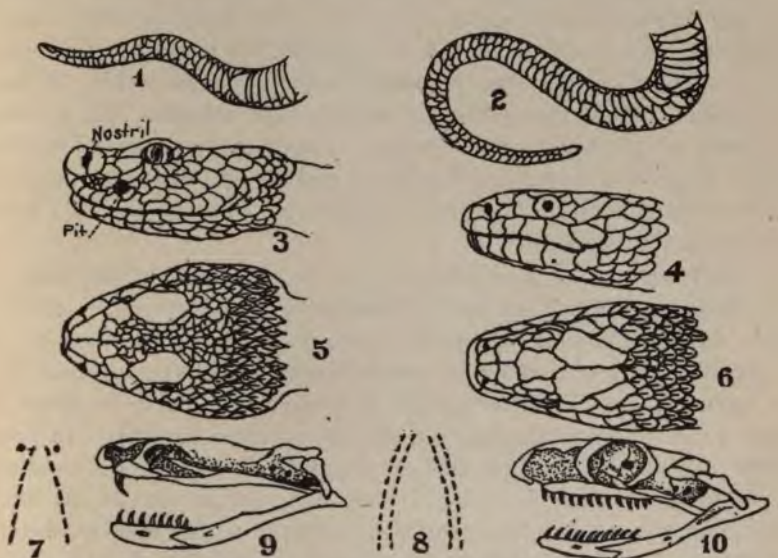


FIG. 83.—1, Single row of scales posterior to vent (poisonous snakes—water moccasin); 2, double row scales of harmless snake (Natrix); 3 and 5, side and dorsal view of head of pit viper; 4 and 6, side and dorsal view of head of harmless snake (Natrix); 7 and 9, bite puncture and skull of Elaps; 8 and 10, bite puncture and skull of harmless snake.

the jaws close and pressure is exerted on the poison glands so that the venom pours out.

Cobra and Krait bites (colubrine snakes) produce more or less similar symptoms such as paralysis of articulation with nausea and vomiting and later paralysis of the respiratory apparatus. There is only an insignificant reaction at the point of bite.

In rattlesnake bites (viperine snakes) there is marked pain at the

site of the wound with much swelling and hæmorrhagic infiltration. The swelling and petechial mottling spread up the limb from the point of entrance of the venom. Cold sweats, nausea, weak heart, and syncope are common.

The most venomous snakes seem to be the sea-snakes (Enhydrina). The Tiger snake of Australia is almost equally venomous and the Krait (B. cœruleus) next. The rattlesnake is about one-fifth as venomous as the Krait.

The nonspecific treatment of snake-bite poisoning is 1. by applying a tight ligature above the site of the bite. The ligature, which should preferably be a rubber band, is to be applied about a single bone extremity, not about one with two supporting bones. 2. The making of deep incisions about the fang punctures and thorough irrigation with a strong solution of potassium permanganate. Rogers has recommended that the punctures be enlarged with a lancet and the resulting wound packed with crystals of permanganate. Chromic acid injections (1%) have also been recommended.

Internally alcohol does not seem to be of any value, in fact many of the deaths have been attributed to excessive ingestion of whiskey. Strychnine in large, almost poisonous doses, was highly recommended in Australia but the statistics seem to make the value of this remedy doubtful.

Antivenins.—The active agents of snake venoms may be either of the nature of hæmorrhagins, neurotoxins, or fibrin ferments. In colubrine snakes the neurotoxin vastly predominates while with the viperines it is the hæmorrhagin. Certain Australian snakes contain all three bodies in about equal proportion while with the rattlesnakes of America it is almost entirely the hæmorrhagin which causes the poisoning. The Elaps of Florida is a colubrine snake and its venom is neurotoxic in nature.

The cause of death in colubrine snake bites is chiefly from paralysis of the respiratory centers while with the Pit Vipers it is chiefly from hæmorrhages in the vital organs. Antitoxins have been prepared against both viperine and colubrine venoms and these are specific, a colubrine antivenin will not be of value against a viperine bite. Antivenins should be administered either intravenously or intramuscularly. The amounts recommended for injections to neutralize a fatal dose of snake poison vary from 100 to 300 c.c. of the antivenin serum. There is no accurate *standardization.*

NOTES ON ANIMAL PARASITOLOGY.

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PART IV.

CLINICAL BACTERIOLOGY AND ANIMAL PARASITOLOGY OF THE VARIOUS BODY FLUIDS AND ORGANS.

CHAPTER XXIII.

DIAGNOSIS OF INFECTIONS OF THE OCULAR REGION.

It is advisable before taking material for cultures or smears to cleanse the nasal area of the eye-lids, and especially about the caruncles, with sterile salt solution. Then, by gently pressing on the lids, we may be able to get pure cultures of the organism causing the infection. Normally, we may find in the region of the caruncles various skin organisms, especially staphylococci, giving white colonies.

The xerosis bacillus and white staphylococci may be considered normal findings in the conjunctival sac. Streptococci and pneumococci have also been reported from apparently normal conjunctival secretions.

A small particle of sterile cotton, wound on a toothpick, with the aid of a sterile forceps, makes an excellent swab for obtaining material for smears; the same may first be drawn over an agar surface in a Petri dish in a series of parallel lines of inoculation before making the smears on slide or cover-glass.

When there is considerable discharge, a capillary pipette, with a rubber bulb, may be used to draw up sufficient material for cultures and smear. Be sure to round off the end of the pipette in the flame and not to use a very fine capillary tube.

In conjunctival cultures, plates of glycerine agar or agar plates smeared with blood are to be preferred, as the gonococcus and Koch-Weeks bacillus will only grow on blood or hydrocele agar. The diphtheria and xerosis bacilli grow well on glycerine agar.

In addition to the white staphylococcus, the streptococcus may be present when inflammation of the nasal duct exists.

The pneumococcus is a fairly common cause of serpiginous corneal ulcerations. Active treatment is necessary.

Corneal ulcerations are not apt to appear even with a pneumococcal conjunctivitis unless there be an injury of the epithelium.

The B. xerosis is possibly a harmless organism and must not be accepted as explaining an infection unless other factors have been eliminated. The true diphtheria bacillus, which the xerosis so much resembles, may cause a pseudomembranous inflammation.

The B. pyocyaneus may cause severe purulent keratitis as well as conjunctivitis. The pyocyaneus toxin appears to be a factor.

The gonococcus and the Koch-Weeks bacillus are usually responsible for the very acute cases of conjunctivitis. Both these organisms are characteristically intracellular and are Gram negative.

The diplobacillus of Morax and Axenfeld is more common in chronic, rather dry affections of the conjunctiva.

In cases of ozena with involvement of the nasal ducts Friedländer's bacillus may be found.

Even in cases without ozena, capsulated, Gram negative bacilli of the Friedländer group have been frequently reported in conjunctival inflammation.

The nodules of the eye-brows give the most convenient area to take material from in the diagnosis of leprosy, either the fluid expressed after scraping or a piece of tissue cut into sections. Conjunctival ulceration in leprosy may show abundant bacilli as is also true of corneal ulcerations. Ordinarily it is impossible to find tubercle bacilli in tuberculous conjunctival discharges.

The discharge from a tuberculous dacryocystitis may show them satisfactorily. Animal inoculation is preferable in the diagnosis of ocular T. B. The pneumococcus is, however, the most important organism in dacryocystitis—rarely the B. coli.

In a gonorrhœal ophthalmia the secretion is much more abundant and there is an absence of contaminating organisms, the reverse of infection with the confusing M. catarrhalis. As a matter of fact, large numbers of M. catarrhalis may be present in the conjunctival secretion with only slight irritation being observable.

In keratomycosis the cause has been ascribed to Aspergillus fumigatus.

Certain fungi of the genus Microsporum have been thought to be the cause of trachoma, as have also certain bacillary forms. One should be very conservative about reporting fungi in smears or cultures of *external surfaces*

The larval stage of Tænia solium (Cysticercus cellulosæ) has a predilection for eye as well as brain. It is usually situated beneath the retina.

The adult Filaria loa tends at times to appear under the conjunctiva or in the subcutaneous tissue of the eye-lids.

Fly larvæ have been reported from the conjunctival sacs in the helpless sick.

Prowazek has thought that certain fine dots within the cytoplasm of epithelial cells, which stain best by Giemsa's method and which he considered protozoal in nature, were the cause of trachoma.

CHAPTER XXIV.

DIAGNOSIS OF INFECTIONS OF THE NASAL AND AURAI CAVITIES.

In taking material from the nasal cavities, for the bacteriologica examination, it is well to wash about the alæ with sterile water an then have the patient blow his nose on a piece of sterile gauze and tak the material for culture or smear from this. If the material is purulen and located at some ulcerating spot, it is best to use a speculum, an either touch the spot with a sterile swab or use a capillary bulb pipett with a slight bend at the end.

Normally, we find only white staphylococcus colonies and colonies o short-chain streptococci. The M. tetragenus, B. xerosis, and Hoff man's bacillus are also occasionally found.

In some cases of ozena we may find an organism of the Friedlände type in pure culture.

Biscuit-shaped diplococci, both Gram negative and positive, ar to be found either normally or in cases of coryza. M. catarrhalis ha probably been frequently reported as the meningococcus. Still, th meningococcus has been found in the nasal secretions of patients wit cerebrospinal meningitis. B. influenzæ and the pneumococcus hav also been frequently found in cultures from the nasal secretions.

Diphtheria involving the nasal cavity must always be kept in mind and in quarantine investigations the examination of the nasal secretio culturally should be a part of the routine.

The tubercle bacillus may be found in nasal ulcerations; it is, how ever, only present in exceedingly small numbers. On the other hand one of the best diagnostic procedures in leprosy is to examine smear from nasal mucous membrane for the B. lepræ. In such ulceration the bacilli are found in the greatest profusion. Rarely glanders ma cause ulcerations.

B. proteus is frequently responsible for the production of foul odor in nasal discharges but does not seem to produce inflammatory con ditions of the nasal mucosa. It simply decomposes the discharges

Various fungi have been reported from the nose, but in such a region the strictest conservatism in reporting should be observed.

Recently sporozoa have been reported in a case of nasal polyp.

So many degenerative changes in epithelial cells resemble protozoal forms that such findings require ample confirmation.

The larval form of Linguatula rhinaria is a rare parasite of the nasal cavities; it is not infrequent, however, in the nostrils of dogs.

Various fly larvæ are far more common, and the "screw-worm," the larvæ of the Chrysomyia macellaria, is common in certain parts of tropical America, and may by its burrowing effects cause fatal results.

The larvæ of Sarcophaga have in particular been found in the nasal cavities of children. Myriapods, while of very little importance elsewhere, have been reported more than thirty times from the nasal fossæ.

In a study of the bacteriology of otitis media, in 277 cases, Libman and Celler found streptococci present alone in 81%, streptococcus mucosus in 10% and the pneumococcus in 8%; staphyococci, B. pyocyaneus and B. proteus have also been found. Mixed infections are common.

The middle ear is normally free of bacteria, but in affections of the throat, as with streptococci, pneumococci, and diphtheria, these organisms may infect it by way of the Eustachian tube.

The moulds are of greater importance in affections of the external auditory canal than the bacteria. The cerumen seems to make a good culture medium so that various species of Aspergillus, Mucor, etc., develop and close the canal. These infections are often introduced by the patient's finger. Various mites and fly larvæ have been reported from the ear.

CHAPTER XXV.

EXAMINATION OF BUCCAL AND PHARYNGEAL MATERIAL.

In a preparation made from material taken by a sterile swab from the region of the normal buccal and pharyngeal cavities and stained by Gram's method we are struck by the variety of organisms present.

Gram positive and Gram negative staphylococci are present, as are also streptococci, pneumococci, leptothrix forms, and very probably yeasts and sarcinæ types with many Gram negative bacilli. If pseudo-diphtheria organisms are present, we have these showing a Gram positive

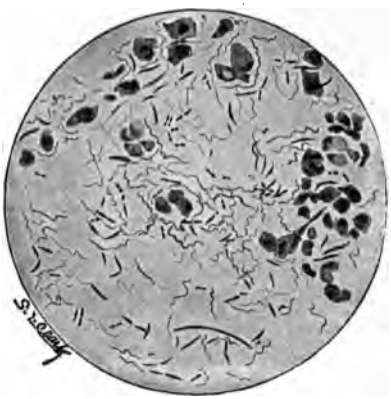

Fig. 84.—Vincent's angina. Spirochæta vincenti. (*Coplin.*)

reaction. If this material is smeared on agar plates and cultured at 37° C., we are struck by the fact that the colonies on the plates may be exclusively staphylococcal and streptococcal.

It is very difficult, if not impossible, to distinguish a pneumococcus colony from a streptococcus one on a plate culture. The presence or absence, however, of the pneumococcus is distinctly shown in the Gram-stained smear, either by its lance-shaped morphology or the presence of a capsule. It has been my experience that smears from *about 15%* of normal individuals show capsulated pneumococci.

In diphtheria examinations we rely chiefly on the cultural findings on Löffler's serum. Where the process is streptococcal or due to the organisms associated with Vincent's angina, the immediate examination of a smear from the suspected spot or area gives greater diagnostic information. The streptococcus being so abundant in cultures from normal throats, it is difficult to determine its significance in a culture; abundance of streptococci in a smear from an ulceration or bit of membrane, however, is of etiological import.

By staining with Neisser's method it is possible to make an immediate diagnosis of diphtheria from a smear from a piece of membrane in about 25% of cases. It is well, however, to always culture such material.

Material from the throat is ordinarily best obtained with a sterile copper-wire cotton-pledget swab. The platinum loop usually bends too easily. A sterile forceps may be more convenient for obtaining particles of membrane. It is believed that ulcerative conditions of the throat, associated with the presence of the large fusiform bacillus and delicate spirillum, which make the picture of Vincent's angina, are more common than is usually so considered. As a rule, only cultures on serum are made and very rarely direct smears. If a smear were always made and stained by Gram's method (with a contrast stain of dilute carbol fuchsin) at the same time the culture was made, it is probable that much information of value would be obtained.

The B. fusiformis is an anaerobe which gives a fetid odor but culturally has no distinct characteristics. The spirillum has not been cultivated. It has been thought that the bacillus and spirillum are different stages of the same organism.

At times aggregations of the fusiform bacillus give the appearance of branching so characteristic of diphtheria organisms. Being Gram negative, however, the differentiation is easily made—the B. diphtheriæ being Gram positive. Again the attenuated ends of the fusiform bacillus are diagnostic.

Direct smears are the procedure of choice in streptococcal and pneumococcal anginas as well as in Vincent's angina.

Unless very familiar with the morphology of Treponema pallidum and using Giemsa's staining procedure, we should be very conservative in reporting such an organism from suspected syphilitic ulcerations of the throat.

The thrush fungus (Endomyces albicans) may be easily demonstrated in a Gram-stained specimen as violet mycelial structures.

Yeasts due to food particles are not infrequently observed in smears and cultures from the mouth.

Actinomycosis may develop about a carious tooth and the finding of the ray fungus in the granules from the pus may give the diagnosis.

Amœbæ and flagellates have been reported from the mouth. Also in the remarkable disease "halzoun," flukes have been found to be the cause of the asphyxia.

In the tropics, round worms may be vomited up and, lodging in the pharynx, may have to be extracted.

During the campaign of Napoleon in Egypt many cases of leech involvement of the nasal and buccal cavities were noted. The parasite was the Limnatis nilotica which gained access to the upper pharynx through drinking water from springs and pools. Many such cases continue to be reported from the Mediterranean basin.

CHAPTER XXVI.

EXAMINATION OF SPUTUM.

FREQUENTLY the material submitted for examination as sputum is simply buccal or pharyngeal secretion, or more probably secretion from the nasopharynx, which has been secured by hawking. It should always be insisted upon that the sputum be raised by a true pulmonary coughing act, and not expelled with the hacking cough so frequently associated with an elongated uvula. When there is an effort to deceive, some information may be obtained from the watery, stringy, mucoid character of the buccopharyngeal material and also from the presence of mosaic-like groups of flat epithelial cells (often packed with bacteria). The pulmonary secretion is either frothy mucus or mucopurulent material, and if the cells are alveolar they greatly resemble the plasma cells. At times these cells may contain blood pigment granules (heart-disease cells).

In the microscopic examination a small, cheesy particle, the size of a pin head, should be selected. This should be flattened out in a thin layer between the slide and cover-glass and should be examined for elastic tissue, heart-disease cells, eggs of animal parasites, amœbæ, and fungi. Echinococcus hooklets, Curschman spirals besprinkled with Charcot-Leyden crystals, and hæmatoidin and fatty acid crystals may also be observed.

Curschman spirals indicate bronchial as against cardiac or uremic asthma. Charcot-Leyden crystals have no special significance.

It may facilitate the examination of the sputum for elastic tissue and actinomycosis and other fungi to add 10% sodium hydrate to the preparation.

To make smears for staining, the sputum should be poured on a flat surface, preferably a Petri dish, and a bit of mucopurulent material selected with forceps. A dark back-ground facilitates picking out the particle. A toothpick is well adapted to smearing out such material on a slide. After using, it can be burned. When dry, the smear is best

fixed by pouring on a few drops of alcohol, allowing this to run over the surface, and then, after dashing off the excess of alcohol, to ignite that remaining on the film in the flame and allow to burn out.

A mark with a grease pencil, about 1/2 inch from the end, gives a convenient surface to hold with the forceps and also prevents the stain subsequently used from running over the entire surface. Sputum should as a routine measure be stained by the Ziehl-Neelson method and by Gram's method.

In examining for tubercle bacilli it may be necessary to employ some method for concentrating the bacterial content of the sputum prior to making the smear. A very satisfactory method is that of Mühlhäuser-Czaplewski. Shake up the sputum with four to eight times its volume of 1/4% solution of sodium hydrate in a stoppered bottle. When the mixture has become a smooth, mucilaginous-looking fluid, add a few drops of phenolphthalein solution and bring the pink mixture to a boil.

Then add drop by drop a 2% solution of acetic acid, stirring constantly, until the pink color is just discharged. If the least excess of acid is added over that just sufficient to cause the pink color to disappear, mucin will be precipitated. Now pour this mixture into a centrifuge tube and smear the sediment on a slide and stain for tubercle bacilli.

Sputum smears stained by some Romanowsky method or by the hæmatoxylin-eosin stain are best adapted for the study of various cells, and in particular of the eosinophile cells so characteristic of bronchial asthma. In sputum from cancer of the lungs the large vacuolated cells may be found.

When examining the sputum of the bronchopneumonia of influenza the formol fuchsin gives the best results. The influenza bacilli are found in little masses, frequently grouped about small collections of M. tetragenus. The cocci stain a rich purplish-red, while the small influenza bacilli take on a light pink color.

Red cells show up well in specimens stained by the Romanowsky method; if rouleaux formation is marked, it may indicate pulmonary infarction.

In culturing sputum a mucopurulent mass should be washed in sterile water and should then be dropped into a tube of sterile bouillon. With a sterile swab it should be emulsified and successive streaks made along the surface of an agar or glycerine agar plate. In obtaining cul-

tures from influenza sputum, first smear the material thoroughly over a blood-serum slant; then inoculate, by thorough smearing over the surface of successive blood-streaked agar slants, the material on the surface of the blood-serum slant. The platinum loop should be transferred from one slant to another without recharging. The influenza bacillus seems to grow better if the blood-streaked agar slants are prepared just before inoculating with the sputum. All that is necessary is to sterilize an ear, puncture and take up the exuding blood with a large loop. Cultures for tubercule bacilli are impracticable. A guinea-pig should be inoculated.

The blood-stained watery sputum of plague pneumonia should be cultured on plates of plain agar and 3% salt agar at the same time. An ordinary smear stained with carbol thionin, however, practically makes a diagnosis.

Pneumococci, M. catarrhalis, and Friedländer's bacillus in sputum are best demonstrated by Gram's method of staining.

The distinct capsule staining of the pneumococci in a Gram preparation of sputum from a suspected case of pneumonia is of value in diagnosis.

The finding of the ray fungus (D. bovis) in sputum gives the diagnosis of actinomycosis.

Moulds, especially Aspergilli, may be found in sputum. Species of Mucor, Cryptococcus, and Endomyces have also been reported.

Amœbæ from liver abscess rupturing into the lung may be found. Very important pulmonary infections are those with Paragonimus westermanii. This is recognized by the presence of operculated eggs in the sputum.

A fluke, F. gigantea, was once found in sputum.

Hydatid cysts, either of the lung or of the liver, rupturing into the lung, may be recognized by the presence of echinococcus hooklets. The material is bile-stained if from the liver.

Strongylus apri has been reported once from the lungs and embryos might be found in the sputum. In pulmonary bilharziosis Schistosoma eggs may be found in the sputum.

CHAPTER XXVII.

THE URINE.

MATERIAL for staining is best obtained by centrifuging the urine, then pouring off the supernatant urine, to replace it with a 1% aqueous solution of formalin. Shaking for a few seconds we again centrifuge, pour off the fluid from the sediment, and make smears from the sediment.

The addition of a loopful of egg albumen or blood serum to about twice that amount of urinary sediment gives better results. (See under Staining Methods.)

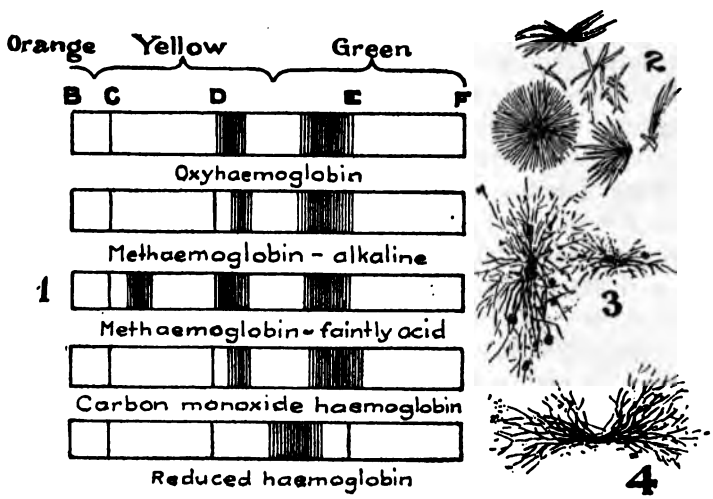

FIG. 85.—1, Various absorption bands of spectrum; 2, crystals of glucosazone (Phenylhydrazine sugar test); 3, Cammidge crystals (interacinar type of pancreatitis); 4, Cammidge crystals (interlobular type of pancreatitis).

The smear may be stained directly by Wright's method or after fixing by heat with Gram's stain, T. B. stain, or hæmatoxylin and eosin. The latter is the best for the staining of epithelial cells and animal parasites; the Gram method for bacteria.

It is frequently difficult to distinguish the spores of moulds from red blood-cells except by measurement and staining reactions. Spores of moulds rarely exceed five microns.

It is difficult to determine the presence of blood in urine in higher dilution than 1 to 300 with the spectroscope. The occult blood test will show it in much higher dilution.

To secure urine for bacteriological examination catheterization is rarely necessary in men—in the case of women it is the proper method.

The glans penis and meatus should be thoroughly washed with soap and water, after which dilute alcohol (50%) should be used. The greater part of the urine first passed should be rejected and only the last portion passed should be caught in a sterile receptacle. A drop of this

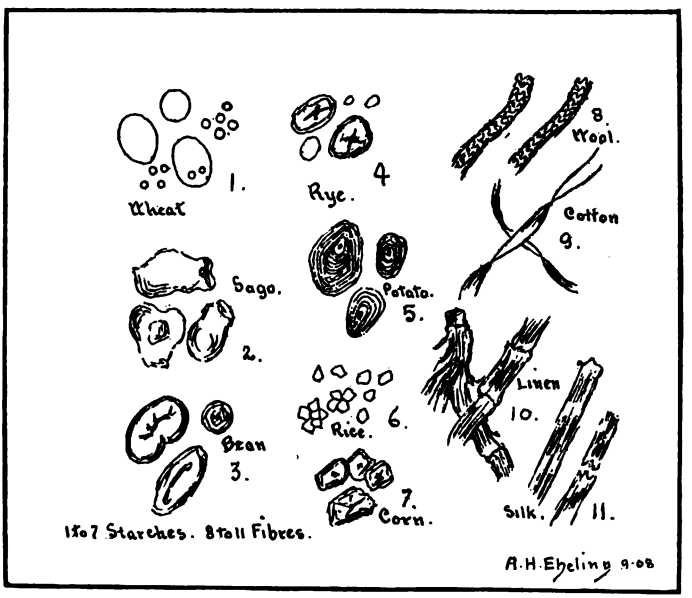

FIG. 86.—Starches and fibers found in urine.

urine may be either streaked over the surface of an agar or a lactose litmus agar plate, or so treated after being first diluted in a tube of sterile bouillon.

The lactose litmus agar medium is very useful in distinguishing typhoid or paratyphoid colonies (blue) from colon, and streptococcus or staphylococcus colonies (pink). The urine may be added to tubes of melted agar and then poured.

Cystitis from a colon infection gives an acid urine; that caused by Proteus vulgaris an alkaline urine.

The old designation B. termo so often employed in connection wit the bacteriology of the urine in older works applied to the proteus grou

The bacillus of plague and Malta fever are also found in the urin

While the smegma bacillus in urine may be differentiated from th tubercle bacillus by the former losing its red color, by prolonged decolori zation with acid alcohol, yet it is chiefly by the subcutaneous inoculatio of the guinea-pig that we should diagnose genito-urinary tuberculosi Inject the sediment after centrifuging. Gonococci are reported fro Gram-stained smears.

Yeasts and moulds frequently contaminate urine, especially diabeti urine, after it has been passed. Amœbæ and flagellates (Trichomona vaginalis in females) may be found in urine. Also the itch mite is no rarely found in the urine of those having scabies of the penis.

Eggs of Schistomum hæmatobium (bilharziosis) are importan diagnostic findings; these are terminal-spined. Those of rectal bilhar ziosis are, as a rule, lateral-spined.

In chylous urine the filarial embryos may be found. This examina tion is facilitated by centrifugalization.

The eggs of the E. gigas may be recognized in urinary sedimen by their pitted appearance.

The vinegar eel may be found in the urine of females who have use vaginal douches of vinegar.

Echinococcus hooklets, scolices, or laminated membrane have bee found in the urine.

The larval dibothriocephalid, Sparganum mansoni, has been reporte three times in urine (urethra).

Oxyuris from the vagina may be found in urine.

Various mites may be found in urinary sediment as the result of lac of care in the washing of the receptacle and are entirely accidental.

Crystals of biliverdin may be found in the urinary sediment in marke jaundice. They somewhat resemble crystals of tyrosin but are brownis in color while those of tyrosin are black. Furthermore, it is excessivel rare to find crystals of leucin and tyrosin in the urinary sediments, an in such diseases as acute yellow atrophy of the liver, the urine should b concentrated to one-tenth its volume and the residue treated with alcoho The tyrosin crystalline sheaves and the leucin striated globules crystalli out from the alcohol.

CHAPTER XXVIII.

THE FÆCES.

It is advisable to examine a stool macroscopically before taking up the microscopical examination. The mucus shreds or casts of the bowel in mucous colitis or membranous enteritis may give the diagnosis of obscure abdominal pain. Stools containing pus may often be noted without the aid of the microscope.

In obstruction of the common bile duct we have alcoholic, whitish, foul-smelling stools. If the putty color be due to bacterial change exposure to the air will restore the brownish tinge.

Sprue stools are white-wash to putty colored, pultaceous, and filled with air bubbles. The amount is excessive.

Fatty stools are best examined microscopically.

As so many solid masses resemble gall-stones it is well to dissolve the suspected mass in hot alcohol and examine for cholesterin crystals upon evaporation of the alcohol.

If the fæcal examination is to be made for the diagnosis of amœbæ, in a case where the characteristic mucous stools are not present, or to verify the existence of flagellates, it is best to give a dose of salts early in the morning and examine the liquid stools which follow such treatment. This treatment is satisfactory for examination for intestinal parasites or ova.

A very practical way of obtaining amœbæ is to pass a rectal tube or a piece of drainage tube with fenestrations into the bowel, and amœbæ may be found in the mucus filling the perforations in the tube.

The blood-flecked mucus of bacillary dysentery or a piece of grayish-brown mucus from a typical amœbic dysentery stool are best suited for cultural examination.

If the purpose of the examination is to determine the digestive power of the alimentary tract for proteids, carbohydrates, or fats, it is best to use a test diet, as that of Schmidt and Strasburger.

Prior to using this test diet, one should familiarize himself with the microscopic appearances resulting from such a diet in a normal person;

information is then at hand to judge of variations from the normal. The examination of the fæces of persons, on ordinary and specifically undetermined articles of diet, is very unsatisfactory when the state of digestion of muscle fibers and the question of fat digestion are at issue.

In examining the fæces of the normal person and likewise with the patient, wait until the second or third day so that the fæces of previous diets may have passed out.

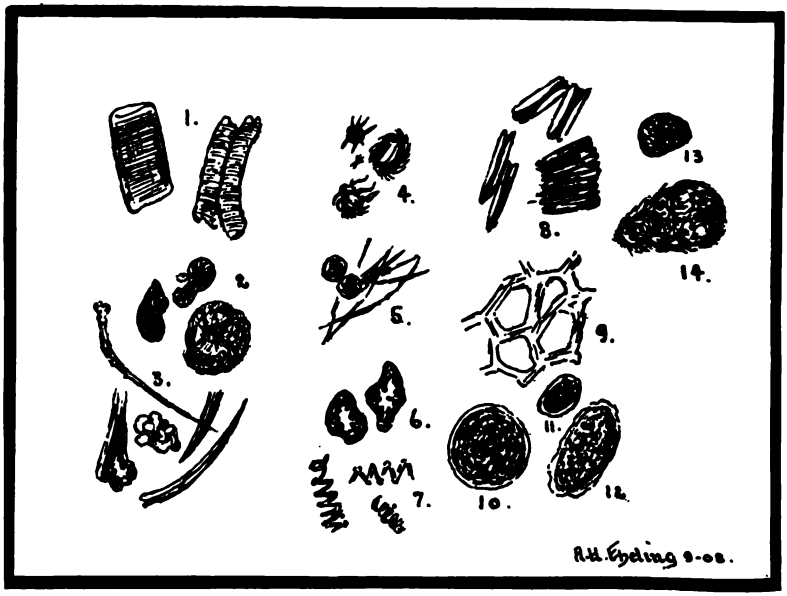

FIG. 87.—Familiar objects in fæces. 1, Muscle fibres; 2, soaps; 3, vegetable hairs; 4, fatty acid crystals projecting from neutral fat globule; 5, soap crystals with leukocytes; 6, stone cells; 7, vegetable spirals; 8, palisade cells from bean; 9, parenchyma of vegetable tissue; 10, 11, 12 and 14, vegetable cells; 13, pollen.

Diet: breakfast, 7 A. M., bowl of oatmeal gruel (40 grams oatmeal, 10 grams butter, 200 c.c. milk, 300 c.c. water). Also one very soft-boiled egg (1 min.) and 50 grams zwieback. In the forenoon, 500 c.c. of milk.

For dinner, 2 o'clock, chopped beef broiled very rare (125 grams with 20 grams butter poured over it). Also a potato puree (200 grams mashed potato, 50 grams milk, 10 grams butter). Also 1/2 liter of milk and 50 grams zwieback.

For supper, 7 o'clock, the same articles as for breakfast.

Having familiarized one's self with the degree of digestion of muscle, starch, and fat in a normal person, we are in a position to judge of the state of assimilation in a patient.

We judge of muscle digestion by the intactness of the striations. If a muscle remnant is only a homogeneous yellowish particle, it shows satisfactory digestion. If it is rectangular, with well-defined cross striations, it shows poor digestion for meat (Azotorrhœa). A loopful of fæces should be smeared into a drop of Gram's solution for starch-digestion determination. Normally there should be no blue-staining starch granules.

Soaps are gnarled bodies everted like the pinna of an ear, while soap crystals are comparatively coarse and do not melt on application of gentle heat as do the more delicate fatty acid crystals. Neutral fat is in round or irregular globules. The best stain for fat is Sudan III (saturated solution of Sudan III in equal parts of 70% alcohol and acetone).

Mix up the fæces with dilute alcohol (50 to 70%) and then add a drop of the above solution and apply a cover-glass quickly. The fat globules show as orange or golden-yellow bodies.

Steatorrhœa, or the presence of fat in abnormal quantities in the fæces, is shown by the pale, bulky, greasy stools as well as in the microscopical examination.

Average for normals in 1 gm. dried fæces:

Total fat,	225 mg.	(22.5%)
Total fatty acid,	86 mg.	(37.9% of all fat)
Total soap,	74.7 mg.	(33.4% of all fat)
Total neutral fat,	64.4 mg.	(28.5% of all fat)

In normal cases the only fat elements recognizable are yellow calcium or colorless soap.

As quantity of fat increases (as say 500 to 600 mg.) droplets of neutral fat appear with or without increase in number of soap masses. Also needles and splinters of fatty acid and soaps appear. Much connective tissue débris shows defect in gastric digestion, as only the stomach digests connective tissue.

In addition to the fæces examination we should check the results from the test diet with indican and nitrogen partition determinations of the twenty-four hour urine specimen—the ratio of ammonia nitrogen

to total nitrogen indicating the functional power of liver and the indican the question of stasis in lower part of small intestine.

Cammidge's reaction (urine) combined with Azotorrhœa and Steatorrhœa makes for a diagnosis of chronic pancreatitis. Mayo Robson states that the Cammidge reaction is almost invariably present in cases of duodenal ulcer and in 70% of cases of Cholelithiasis, in both of which diseases we have duodenal catarrh.

In examining a liquid stool after salts, it is well to color the drop of fæces, which is to be covered with the cover-glass, with a small loopful of 1/2% solution of neutral red. If diluting fluid is used, it should be salt solution, and not water. The neutral red tinges the granules of the endoplasm of amœbæ and flagellates a very striking brown-red color, thus differentiating them from vegetable cells or body cells.

Whether examining the thin fæces or the mucus particle, it is well to reserve report on amœbæ or flagellates until motion is observed. Encysted protozoa are difficult to diagnose.

When a smear preparation is desired, we may smear out a fragment of mucus and stain by Romanowsky's or Gram's method. The character of the bacteria present appears to be of diagnostic value—especially in the case of infants and young children. Beautiful preparations may be made by mixing the fæces with water, then centrifuging for one minute. This throws down vegetable débris and crystals. Now decant the supernatant fluid, which holds the bacteria in suspenion, and add an equal amount of alcohol. Again centrifuge, decant, and smear out and examine the bacterial sediment.

Simply taking a small mass of fæces and emulsifying it with a wooden toothpick on a concave slide in 70% alcohol—then, after the sediment settles, taking up a loopful with platinum loop from the surface and smearing out, gives a very satisfactory smear. Gram's method, with dilute carbol fuchsin counterstaining, gives the best picture.

The Boas-Oppler bacillus may be found in the stools in this way. Normally, a Gram-stained stool shows a great preponderance of Gram negative bacilli and such a finding in a measure excludes cancer of the stomach. Organisms which are Gram positive as well as the Boas-Oppler bacillus are, 1. Lactic acid bacilli—these show Gram negative areas in the slender bacilli. 2. A type of bacillus similar to the Colon bacillus but Gram positive and noncultivable (found in acid stools). 3. Bacilli of the B. subtilis type.

It is very important to examine the fæces for T. B. With children a diagnosis of tuberculosis may be made in this way when the sputum cannot be obtained, the pulmonary secretion being swallowed. The preparation on the concave slide as described above should be stained for T. B.

To culture for typhoid, dysentery, cholera, or other bacteria, take up the material in a tube of sterile bouillon and smear it out with a swab over a lactose litmus agar plate or an Endo or Conradi-Drigalski plate. Before streaking the plates they should be very dry on the surface. This can be best done by pouring into a plate with a circular piece of filter-paper in the lid and placing in the incubator for one-half hour to dry. The filter-paper absorbs the moisture. Then inoculate the surface of the plate with the fæcal material.

Müller's method for pancreatic functioning determination is to give a calomel purge two hours after a meal. A little of the liquid stool is smeared on the surface of blood-serum and the tube incubated at 60° C. (paraffin oven). If the surface is smooth, no trypsin was present; if dotted with spots of digestion liquefaction, it shows that the pancreatic secretion is present. This should be tried with the Cammidge reaction of the urine.

Epithelial cells are generally more or less disintegrated. In the mucus of bacillary dysenteric stools, however, large intact phagocytic cells are frequent, which may be mistaken for encysted amœbæ.

Triple phosphate crystals are frequently observed in fæces, as may also be crystals of various calcium salts. Charcot-Leyden crystals are rather indicative of helminthiases.

Various flagellates, and in particular Lamblia, may be responsible for diarrhœal conditions which may cause rather serious symptoms.

Balantidium coli has been reported several times as the cause of dysenteric conditions. Coccidiadea are found in the fæces.

In the Philippines the Entamœba histolytica is the most important of the animal infections. Besides examining for it in a cover-glass preparation, we should attempt to make cultures. A diluted bouillon, 1 : 10, containing 1 1/2% agar, with a reaction about —1.5, is a satisfactory medium. The most important points in success seems to be proper symbiosis and proper reaction. In a series of four tubes containing the same media, but with —2.5, —2, —1.5, and —1, the amœbæ may only grow in one of the tubes. Walker recommends smearing a cover-glass

with suitable agar, then inoculating the surface, we invert it over the hollow of a concave slide, sealing the margins with vaseline. This enables us to study development with a high power of the microscope.

For culturing amœbæ, whether from fæces, water, or vegetable washings, it is convenient to add the material to either diluted bouillon, Musgrave and Clegg's medium, or the Smith's glucose medium.

It is exceedingly difficult to obtain cultures of pathogenic amœbæ in fluid cultures while this procedure is almost invariably successful when examining water supplies. In fact certain authorities have stated that the cultivation of a flagellate or amœba from the stool indicates that the protozoon was a harmless commensal. Musgrave and Clegg used plates, the surfaces of which had been dried, and smeared a very small quantity of the material on the surface for the reason that if bacterial growth were luxuriant the amœbæ failed to grow.

It is in the fæces we examine either for the parasites or for their ova in connection with practically all the flukes, except the lung fluke and the bladder fluke; for intestinal tæniases and for practically all the round worms, except the filarial ones.

Bass has recommended that fæces which have been made fluid be centrifuged and the supernatant fluid containing vegetable débris be poured off. The sediment contains hookworm eggs. Then pour on sediment a calcium chloride solution of sp. gr. 1050. Again centrifuge and decant. Next add calcium chloride solution of a sp. gr. of 1250 and centrifuge. This brings to the surface the hookworm eggs which may be pipetted off. As a rule, the finding of hookworm eggs is very easy without such a technic.

In the tropics, the examination of the fæces vastly exceeds in value that of urine and is possibly more important than blood examinations.

The larvæ of various insects may at times be detected in the stools, as well as certain acarines (cheese mites, etc.).

The test for occult blood is indicated in helminthiases as well as in the conditions for which it is usually tested.

CHAPTER XXVIII.

BLOOD CULTURES AND BLOOD PARASITES.

CLINICALLY, the most important examination of the blood for parasites is for the presence of various bacterial infections and for certain blood protozoa and also filarial embryos.

The modern method of culturing blood, especially for the detection of typhoid or paratyphoid bacilli, is by the use of the bile media of Conradi. Test-tubes are filled with 7 to 10 c.c. of 1% peptone ox bile, or ox bile alone, and the medium is sterilized in the autoclave. It is good practice to place the syringe in a plugged test-tube containing salt solution, with the needle unscrewed. After autoclaving, the sterile syringe can be taken to the bedside in the test-tube. Using a wide test-tube, a forceps can be sterilized at the same time and used to attach the needle to the barrel of the syringe.

The skin should be scrubbed gently with green-soap solution and water for about three minutes. The skin of the area to be punctured should then be sterilized by the gentle application of Harrington's solution (not scrubbed) for one-half minute, and should then be washed with sterile water. It appears to be safe to simply scrub the area with 70% alcohol for one or two minutes. Applications of pure carbolic acid on a gauze wad for a few seconds followed by neutralization with 70% alcohol gives satisfactory sterilization. A tourniquet is now applied to distend the vein, and the needle is inserted in the direction of the venous flow. Withdrawing 5 to 10 c.c. of blood, we loosen the tourniquet, (otherwise the blood may flow from the puncture) then withdraw the needle, and force out about 1/2 c.c. into the first bile tube, about 1 c.c. into the second, and 2 or 3 c.c. into the third. It is well to reserve some of the blood for Widal tests.

The bile tubes are now incubated for ten to twelve hours and then transfers are made to bouillon tubes. These bouillon tubes can be used in six to eight hours for testing the organism against known typhoid or paratyphoid sera. Test-tubes containing 10 c.c. of ordinary bouillon with 1% of sodium citrate are as satisfactory as bile media.

Some prefer a 2% sodium glycocholate in bouillon while others use a 2% solution of ammonium oxalate in bouillon for blood cultures.

Some prefer to streak plates of lactose litmus agar with material from the bile tubes instead of inoculating the bouillon tubes. Contamination with staphylococci or the presence of staphylococci, streptococci, or plague bacilli in septicæmic conditions show easily accessible colonies.

Schotmuller adds 1 to 3 c.c. of blood to liquefied agar at 45° C., and after mixing pours into plates. The standard method formerly was to add the blood to an excess of bouillon (1 to 5 c.c. of blood to 100 c.c. or more of bouillon). By using the bile media, we can take the blood from the ear in typhoid cases, if preferred. Then if chance staphylococcic contamination occurs, such colonies are readily differentiated from typhoid ones by the pink color on lactose litmus agar. culturing blood in septicæmic conditions, the blood should always be drawn from the vein.

Typhoid cultures are best obtained in the first week of the disease, after that time the Widal is the test of preference.

If a paratyphoid serum is not at hand for testing, it may suffice to inoculate a glucose bouillon tube; gas production indicates paratyphoid. This test should be applied when a very motile organism does not show agglutination with a known typhoid serum. Anthrax and glanders should be considered in blood cultures.

In Malta fever it must be remembered that colonies do not show themselves for several days. Addition of blood to melted agar is a good procedure.

The examination of the blood for the parasites of malaria, filariases, kala-azar, and spirillum fevers has been discussed under their respective headings.

Trypanosomes from human trypanosomiasis have not as yet been cultured, and smears from gland juice or cerebrospinal fluid seem more satisfactory to examine than blood smears unless the blood is taken in 5 to 10 c.c. quantities and centrifuged in sodium citrate salt solution.

The latest method in the diagnosis of trichinosis is to take 5 to 10 c.c. of blood from a vein at the time of the migration of the embryos to the muscles (10 to 20 days). This is forced out into a centrifuge tube containing 3% acetic acid, and the sediment examined for trichina larvæ.

CHAPTER XXX.

THE STOMACH CONTENTS.

FROM a microscopical stand-point there is comparatively little that is of value in the examination of the gastric contents; there is nothing very specific about the findings.

A test meal is not a necessity as in the chemical examination, but either vomitus or material withdrawn with a stomach-tube two or more hours after an ordinary meal suffice.

The microscopical diagnostic points in connection with distinguishing cancer of the stomach from nonmalignant dilatation are: 1. Fragments of cancer tissue. These are very rarely found and are most difficult to diagnose. 2. The presence of flagellates in the early stages of cancer (the so-called anacid stage preceding the development of lactic acid). As flagellates prefer an alkaline medium, they disappear after the acidity due to lactic acid comes on. 3. The presence of the Boas-Oppler bacillus. There are probably several organisms so designated. They are lactic acid producers and are characterized by being very large bacilli ($7 \times 1\mu$) and arranged in long chains which stretch across the field of the microscope. They are Gram positive and do not form spores. They can be cultivated on media rich in blood and are aerobic. They should only be reported when present in great abundance and in long chains. Heinemann thinks it probable that the Boas-Oppler bacillus, Leptothrix buccalis, and B. bifidus may be identical with B. bulgaricus (see under Milk). 4. The absence of sarcinæ and yeasts. The presence of these sarcinæ and fungi in vomitus is indicative of a simple dilatation.

In chronic gastritis the picture of mucus entangling large numbers of epithelial cells is characteristic.

In examining the sediment from the filter-paper after filtering off the stomach contents always use a dilute Gram solution (about 1 to 4) for mounting the sediment. Muscle fibers, yeast cells, red blood cells, and epithelial cells are stained a golden yellow. Starch granules are stained blue while fats are unstained and show as globules of varying sizes.

CHAPTER XXXI.

EXAMINATION OF PUS.

PUS may be collected for examination either 1. with a platinum loop, 2. with a sterile swab, 3. with a bacteriological pipette or 4. with a hypodermic syringe.

It is always well to make a smear and stain it by Gram's method at the same time that cultures are made. The Gram stain gives information as to the abundance of organisms in the pus and as to the probable findings in the culture. Pneumococci and streptococci are differentiated from the staphylococci in this way without the necessity of more or less extended cultural methods.

When autogenous vaccines are to be made, the isolation of the exciting organism is necessary. This is best done by streaking the pus, taken up with a sterile swab and emulsified in a tube of bouillon, over the surface of an agar plate. Practically as convenient and providing a more nutritious medium is to smear the material on a loop or swab over the surface of a blood-serum slant, then to inoculate a second tube from the first without recharging the loop or swab, and so on until three or four tubes are inoculated. Isolated colonies should be obtained in a third or fourth tube.

In examining blood-serum slants inoculated with purulent material, always examine the water of condensation for streptococci.

A bacteriological pipette is very useful when pus is to be sent to a laboratory; the tip can be sealed in a flame and the cotton plug at the other end insures the noncontamination of the contents. The material may be drawn up either with the mouth or with a rubber bulb.

The hypodermic syringe is very useful in puncturing buboes, etc., especially in plague. A small pledget of cotton on a toothpick dipped into pure carbolic acid and touched to a spot over the bubo, which after about thirty seconds is soaked with alcohol, makes a sterile anæsthetic spot at which to introduce the needle of the syringe. It must be remembered that when plague buboes begin to soften, the plague bacilli may be replaced by ordinary pus organisms.

It is remarkable how frequently we get pure cultures from abscess material. In purulent material from abdominal abscesses we are apt to obtain mixed cultures, especially the colon bacillus and B. pyocyaneus, in addition to ordinary pus organisms.

When it is a question between streptococci and pneumococci, it is well to inoculate a mouse; the capsulated pneumococci at the autopsy make the diagnosis.

Animal inoculation is also necessary in plague and glanders, and possibly anthrax. When tetanus is suspected, it should be examined for as described under Tetanus. Tuberculosis should also be identified by inoculating a guinea-pig, as well as by acid-fast staining and culture, if there is any doubt as to the nature of the material.

The black or yellow granules of madura foot, as well as those of actinomycosis, should be examined as recommended in the section on fungi.

Amœbæ, coccidia, and larval echinococci may be found in purulent material, as may also various other animal parasites, as fly larvæ, sarcopsyllæ, etc.

The pus from an amœbic abscess of the liver is as a rule sterile when cultured.

The examination at the time of operation or exploration frequently shows an absence of amœbæ as well as of bacteria. Two or three days later amœbæ may be found in the pus draining from the abscess cavity.

Flukes, lumbricoid, and whip-worms may as a result of their wandering from the intestinal lumen cause abscesses.

Serious ulcerations may follow infection with the Guinea-worm.

CHAPTER XXXII.

SKIN INFECTIONS.

CULTURAL methods are to be preferred in the bacteriological examination of the skin.

This is best done by washing the surface to be examined with soap and water, in order to eliminate chance organisms which may have settled on the surface of the skin in dust or as a result of contact with material containing them. Scrapings are then made with a sterile dull scalpel, and this material is emulsified in a drop of sterile water in the center of a Petri dish. A tube of melted agar at 42° C. is then poured on the inoculated drop and, by mixing, the bacterial flora is distributed over the entire surface of the plate. Of the colonies developing on such plates probably 80% will be found to be staphylococci, and of these the greater proportion will be staphylococci showing white colonies.

Occasionally the aureus or citreus may be isolated.

Streptococci and colon bacilli are rarely found.

The staphylococcus pyogenes aureus is the organism usually isolated from furuncles, circumscribed abscesses, and carbuncles.

Streptococci are the organisms to be expected in phlegmonous infections.

Cold abscesses, which are frequently due to tuberculous infection, are, as a rule, sterile.

Acne pustules may show staphylococci or the microbacillus of acne may be present.

The Bacillus acnes is a short broad bacillus often showing a beaded appearance when stained by Gram's method. It is Gram positive. According to Hartwell it grows readily on glucose agar when cultivated anaerobically (Wright's method). Colonies appear in four to five days.

The bottle bacillus, which morphologically resembles a yeast, is considered to be the cause of dry pityriasis capitis. It may also be found in the comedones of children.

In the tropics, an organism which at times produces lesions similar

to impetigo and again pemphigoid eruptions and at other times widespreading erysipelatous conditions gives cultural characteristics similar to S. pyogenes aureus. It is probably only a virulent aureus. It has been described under the name of Diplococcus pemphigi contagiosi.

The Staphylococcus epidermidis albus, or stitch abscess coccus, is considered by Sabouraud to be the cause of eczema seborrhoicum.

It is in scrapings from the skin of lepromata that we find acid-fast organisms in the greatest profusion. In tuberculosis of the skin the tubercle bacilli are exceedingly scarce. Inoculation of a guinea-pig will probably give positive results with the tubercle bacillus. The leprosy bacillus is noncultivable and noninoculable for experimental animals.

Anthrax and glanders cause skin lesions which can only be surely diagnosed culturally or by animal inoculation.

Plague bacilli may be isolated from the primary vesicles appearing at the site of the flea bite.

Tropical phagedæna is thought by some to be due to a sort of diphtheroid organism.

The skin diseases due to fungi are discussed under that section. Of the skin affections caused by animal parasites, ground itch is the most important. This is a form of dermatitis due to the irritation set up by the hook-worm larvæ penetrating the skin of the foot and leg.

The Sarcopsylla penetrans or jigger (sand flea) is an important agent in ulcerations about the foot.

Certain acarines cause skin lesions, as is also the case with the larvæ of certain flies.

The itch mite (Sarcoptes scabiei) is an important animal parasite of the skin.

The various lice, fleas and bed-bugs are well understood as causes of skin irritation.

Filarial infections are also important especially the ulcers of the Guinea-worm, Calabar swellings of F. loa, the cystic tumors of F. volvulus and the varicose groin glands and elephantiasis of F. bancrofti.

Leeches, as H. ceylonica, may cause serious ulceration.

Oxyuris may cause a severe irritation about the region of the groin and inner surfaces of the thigh, and especially about the vulvar region of female children.

Gnathostomum siamense, a nematode with two lip-like structures

and spine-like structures covering its anterior one-third, has been found once in a tumefaction of the breast.

Plerocercoid larvæ of Dibothriocephalidæ have been found in the subcutaneous tissues.

Certain skin diseases, as Oriental sore and yaws, are protozoal in origin.

CHAPTER XXXIII.

CYTODIAGNOSIS.

THIS method of diagnosis is chiefly employed in the examination of cellular sediments of pleural, ascitic, and cerebrospinal fluid.

The fluids which pathologically collect in the serous cavities are divided into two classes, 1. the transudates, which form as the result of some circulatory inadequacy and 2. the exudates, which result from inflammatory processes.

Transudates have little or no fibrin and very few cellular elements and do not contain nucleo-albumin. Exudates contain nucleo-albumin and usually have a specific gravity above 1018, while that of the transudates is lower than 1018.

There are two simple methods for differentiating transudates and exudates. Moritz adds 2 drops of a 5% solution of acetic acid to the fluid to be tested. A heavy, cloud-like precipitate shows the fluid to be of inflammatory origin (an exudate). A transudate may produce a slight opalescence. Rivalta's test consists in dropping a drop of the fluid to be tested into a cylinder containing 2 drops of glacial acetic acid in 100 c.c. distilled water. A nebulous cloud as the drop of fluid sinks shows an exudate.

For pleural fluids we should receive the material in centrifuge tubes about one-fourth filled with 2% sodium citrate salt solution. This prevents clotting. Having thrown down the sediment, the supernatant fluid is poured off, and in its place a 1% aqueous solution of formalin is added. After mixing and allowing to stand for about five minutes, centrifugalization is again repeated and, pouring off the supernatant formalin solution, we make smears from the sediment. This is either stained by a Romanowsky method or, after fixing with heat (burning alcohol), the smear is stained with hæmatoxylin and eosin.

At the time of securing fluid for cytodiagnosis, cultures should be made on blood-serum for various pyogenic bacteria and, if tuberculosis is suspected, inoculation of a guinea-pig is indicated.

The interpretation of cellular sediments is more difficult than many

books would indicate, there being many factors which tend to complicate the findings. The following are the leading differentiations:

1. A smear showing almost entirely lymphocytes with a few red cells and very rarely a polymorphonuclear indicates a tubercular process.

2. Where a pyogenic process is engrafted on a tuberculous one, we have still the red cells, some degenerated lymphocytes, and in particular polymorphonuclears showing fragmentation of their nuclei.

3. When a hydrothorax results from chronic heart or kidney disease, the characteristic cell is the endothelial cell, which greatly resembles a large mononuclear.

4. Some authorities consider that the cancer cell can be recognized by its occurring in masses and having a markedly vacuolated cytoplasm. It has been claimed that they contain glycogen by which means we can distinguish them from endothelial cells which they so much resemble.

Jousset introduced inoscopy as a means of diagnosing tuberculosis. The fluid was allowed to coagulate and was then digested with an artificial gastric juice. The digested material was then centrifuged and the sediment examined for tubercle bacilli. This process does not seem to have met with much favor in this country. (Using sodium citrate obviates the necessity for digesting the coagulum.)

The same points will hold for ascitic fluid as for pleural fluid.

In taking cerebrospinal fluid for culture and cytodiagnosis we use a stout antitoxin needle without attaching a syringe. Aspiration is responsible for many of the ill effects of lumbar puncture. The needle should be about 4 inches long for an adult. Sterilize the skin and needle as described for blood cultures from a vein. To make a lumbar puncture, place patient on left side with knees drawn up. A line at the level of the iliac crests passes between the third and fourth lumbar vertebræ. Select a point midway between the spinous processes of these lumbar vertebræ and enter the needle 2/5 of an inch to the right of this point, pushing the needle inward and upward. Collect the material in a sterile test-tube. Make cultures on blood-serum and then centrifugalize and examine the sediment as for pleural fluids.

In general terms it may be stated that:

1. A lymphocytosis indicates a tuberculous process.

2. An abundance of polymorphonuclear and eosinophilic leukocytes indicates a meningococcic or pneumococcic infection.

A method of examination considered by neurologists as of differ-

ential diagnostic value is to count the number of cells in a cubic millimeter of the cerebrospinal fluid. The technic is to use a gentian-violet-tinged 3% solution of acetic acid. This is drawn up to the mark 0.5, and the cerebrospinal fluid is then sucked up to 11. After mixing, the cell count is made with the hæmocytometer. Normally we have only one or two cells per cubic millimeter, but in tabes or general paresis this is increased to 50 or 100 cells (greatest at onset of disease).

Trypanosomiasis gives a cellular increase very similar to syphilis.

In the work of the French Sleeping Sickness Commission five cells per cubic millimeter was taken as normal.

CHAPTER XXXIV.

RABIES AND VACCINIA.

RABIES is a disease of dogs and wolves, but is communicable to man and domesticated animals. The virus, whatever it may be, resides in the saliva and nervous structures. It is destroyed by a temperature of 50° C. In man the period of incubation is usually from three weeks to three months, but may be shorter or may extend over one year.

Bites about the face and those with marked lacerations are particularly serious. Bites of rabid wolves give about four times as great a mortality as those of dogs. In the dog there are two types of the disease—dumb rabies and furious rabies.

By inoculating rabbits subdurally with an emulsion of the brain or spinal cord of a rabid animal, and successively the medulla of this rabbit subdurally into other rabbits, we finally so increase the virulence of the infection that rabbits die in six days. Beyond this it is impossible to increase the virulence and it is termed "fixed virus." The pathogenic power of this virus is also changed so that it is not apt to cause rabies if injected subcutaneously. To attenuate this virus the spinal cord of the rabbit is removed and is dried over caustic potash at a temperature of 23° C. The cord is divided into segments about 1 inch in length. Drying for about fifteen days seems to entirely destroy the virus.

To prepare the material for prophylactic injections a small portion of the cord is emulsified with normal salt solution and injected subcutaneously. The German method is to commence with a cord that has been desiccated only eight days. At first injections are given daily, and it is possible to inject three days' cords by the sixth day. The immunity is "active" and the immunizing agent is a "vaccine." Like vaccine virus the product can be preserved (for probably a month) by the use of glycerine so that it is now possible to send the material for inoculation from the laboratory preparing it.

The treatment lasts for about twenty days. In the diagnosis of rabies in dogs it is preferable to preserve the animal so that the development of the symptoms may be observed. In case the dog has been

killed, it may be possible to make a diagnosis by means of the Negri bodies. These are round or oval bodies from 1 to 20μ in diameter, which may be found in the nerve-cells, especially those of the cornu ammonis (Hippocampus major).

These bodies were first described by Negri in 1903. In street rabies large amœboid forms from 18 to 23μ may be found, while in the nerve tissues of animals with "fixed" virus only minute forms, .5μ or less, may be detected. The fact that the virus will pass through a Berkefeld filter is no argument against their protozoal nature. Calkins considers them to be of rhizopod affinity. The term Neuroryctes hydrophobiæ has been given them. They may be demonstrated by staining smears of gray brain substance by some Romanowsky method, especially by the Giemsa stain. The smears should be made by mashing the thin slice of gray matter taken from 1. Cornu ammonis, 2. Region of fissure of Rolando —in dog crucial sulcus—or 3. Cerebellum, with a cover-glass against the slide. Afterward the cover-glass is gently drawn along the slide. As their relation to the nerve-cell is more or less disturbed by such a method, it is preferable to fix brain tissue from the region of the cornu ammonis for five to seven hours in Zenker's fluid, then to imbed in paraffin and make sections. These are stained with Giemsa's stain and the Negri bodies are brought out as lilac-red bodies in the blue cytoplasm of the nerve-cells. It is necessary to differentiate in 95% alcohol.

In the Lentz method the 3μ sections, after removal of the paraffin, are flooded with absolute alcohol. They are then stained with a 1/2% solution of eosin in 6% alcohol for one minute. Wash in water and next stain for one minute in Löffler's methylene blue. Again wash in water. Apply Lugol's solution to the section for one minute and then differentiate in methyl alcohol and water until the section is pink. After washing in water, again stain with Löffler's blue for one-half a minute, then wash in water and dry carefully with filter-paper. Now differentiate in alkaline alcohol (1 drop of a 5% solution NaOH in 30 c.c. absolute alcohol) until the section is pink, then quickly differentiate in acid alcohol (1 drop 50% acetic acid in 30 c.c. absolute alcohol). Pass through alcohols and xylol and mount in balsam. The Negri bodies show as light carmine pink bodies on the light blue ground of the ganglion cells. In the interior of the pink bodies dark blue dots or rings may be observed.

This method can also be used for brain smears.

In addition to examining for the Negri bodies, a rabbit may be inoculated subdurally with a sterile salt-solution emulsion of the medulla of the dead dog.

If the brain or cord of the dog are to be sent to a laboratory for examination they should be packed in ice or placed in glycerine. Take of glycerine one part and one part water. Sterilize the diluted glycerine by boiling, allow to cool, and drop the pieces of brain tissue into this. This does not kill the virus.

Antirabic serum has been prepared by injecting sheep with emulsions of rabbits' cord and brain—at first intravenously, then subcutaneously.

The thorough cauterization of the dog-bite wound with pure nitric acid, as soon as possible after the bite, is imperative even when the Pasteur treatment can be given later.

Vaccinia is a disease produced artificially by the injection of vaccine virus obtained from the calf. The material for vaccine is taken ripe from vesicles about one week after the inoculation. The most potent material is in the pulp at the base of the vesicle and not in the lymph which exudes from the vesicle. The pulp is ground up and mixed with an equal amount of glycerine, which acts not only as a preservative but as a mild antiseptic for nonsporing bacteria. The calves are autopsied after the pulp has been curetted from the inoculated skin of the abdomen to be sure that no disease exists in the calf. The virus is afterward tested for pus organisms, tetanus, and foot and mouth disease.

Guarnieri in 1892 first observed small bodies near the nucleus of infected epithelial cells. He called them Cytoryctes vacciniæ. Calkins regards these bodies as well as the Negri bodies as being rhizopods and the distributed chromatin as idiochromidia (granules of nuclear chromatin within the cytoplasm).

MISCELLANEOUS NOTES.

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APPENDIX.

A—PREPARATION OF TISSUES FOR EXAMINATION IN MICROSCOPIC SECTIONS.

1. **Fixation:**

a. It is most important that the tissues to be examined be placed in the fixing fluid as soon after death or operation as possible. Degenerative changes are in this way avoided.

b. The piece of tissue to be fixed must not be too large. Using a sharp scalpel, or preferably a razor, a slab of tissue about one-half an inch square and not more than one-fifth of an inch thick should be dropped into the bottle containing the fixative. The bottom of this bottle should have a thin layer of cotton with a piece of filter-paper covering it. There should be at least twenty times as great a volume of fixing fluid as of tissue to be fixed. Delicate tissues, as pieces of gut, should be attached to pieces of glass, wood, cardboard, or blotting paper before being placed in the fixative.

c. The most convenient fixative for the average medical man is a 10% solution of ordinary commercial formalin (4% of formic aldehyde gas), either in water or, preferably, in normal salt solution. Fixation is complete in from twelve to twenty-four hours. By placing in the incubator, at 37° C., two to twelve hours in the formalin solution suffices. If fixed in the paraffin oven (56° C.), fixation is accomplished in about one-half hour.

Formalin once used for fixation must be thrown away.

The fixative which probably gives the best histological pictures and with which we obtain the most satisfactory hæmatoxylin staining is Zenker's fluid. This is Müller's fluid containing 5% of corrosive sublimate. It also contains 5% of glacial acetic acid, which latter is only added just before we are ready to fix the piece of tissue. Müller's fluid is:

Pot. bichromate,	2.5 grams.
Sod. sulphate,	1.0 grams.
Water,	100.0 c.c.

Zenker's fluid fixes in about twenty-four hours. After all corrosive sublimate fixatives we should wash the tissues in running water for twelve to twenty-four hours. The precipitate of mercury in the tissues is best gotten rid of by treating the section on the slide with Lugol's solution, rather than the tissue in bulk with iodine alcohol.

In Orth's fluid we add 10% of formalin to Müller's fluid (recommended for nerve tissue).

A saturated corrosive sublimate solution in salt solution with the addition of 5% of glacial acetic acid may be used as a substitute for Zenker's fluid.

2. **Dehydration.**—After washing for twelve to twenty-four hours in running water, following corrosive sublimate fixation, or simply washing for a few minutes after formalin, the tissues should be placed in 70% alcohol. They may be kept in this indefinitely If they are to be sent to a laboratory for sectioning, it is advisable to moisten a pledget of cotton in 70% alcohol and fill in the bottom of the bottle with it. Then drop in the tissues and pack in gently over them sufficient 70% alcohol-saturated cotton to fill up the bottle. All the alcohol should be absorbed by the cotton so that if the bottle should break in transit there would be no damage from the alcohol. The stopper of the bottle should be paraffined or sealed with wax.

Tissues may be left in the 70% alcohol twelve to twenty-four hours and should then be transferred to 95% alcohol for an equal time. They are then transferred to absolute alcohol, where they remain from two to twelve hours and are then placed in xylol. The time in xylol should be as short as possible. So soon as the tissue looks clear it should be removed—thirty minutes to two hours.

3. **Imbedding.**—The tissue is now transferred to melted paraffin. Paraffin melting at 48° C. for winter work, and that melting at 54° C. for summer is to be recommended. The time in the paraffin should not be prolonged. Two hours will ordinarily suffice. Some leave in the paraffin for twelve to twenty-four hours.

Next take a paper box (made of stiff writing-paper folded over a square of wood) and fill with the melted paraffin. As quickly as possible drop in the piece of tissue taken out of the paraffin bath with heated forceps and, so soon as the paraffin begins to solidify on the surface, place the paper box in ice water. When paraffin is rapidly cooled, crystallization is less.

The Acetone Method.—Take the tissues out of the 70% alcohol and place in acetone. After remaining in acetone for one to two hours, the tissues should be transferred to fresh acetone for an equal length of time. Dry calcium chloride in the bottom of the acetone bottles keeps it dehydrated. They should then be placed in xylol for about one-half hour and then embedded in paraffin as directed above.

The Chloroform Method.—The procedure may be the same as in the method of passing through alcohols to xylol, substituting chloroform for xylol and then transferring to paraffin.

Where absolute alcohol is not obtainable, very satisfactory results may be obtained by transferring to a mixture of 95% alcohol and chloroform after immersion in 95% alcohol. Then going from the alcohol-chloroform mixture to pure chloroform, thence to paraffin.

When a piece of tissue is not more than one-fourth inch square and one-eighth inch thick, it is very easy to run it through in three to six hours. Thus:

10% Formalin (in 37° C. incubator),	1 hour.
70% Alcohol (in 37° C. incubator),	1 hour.
95% Alcohol (in 37° C. incubator),	1 hour.
Absolute Alcohol (in 37° C. incubator),	1/2 hour.
Xylol (in 37° C. incubator),	1/2 hour.
Paraffin (in 55° C. incubator),	1/2 to 2 hours.

It is preferable to have a good microtome. The best is that of Minot. Very

satisfactory sections can be cut with the various types of student microtomes, costing from $12 to $20.

(In using a hand microtome, a razor with a flat edge is necessary. After experience, sections thin enough for histological but not for bacteriological examination can be made.)

If the piece of tissue is properly dehydrated and imbedded, thin sections (3 to 10μ) should be easily obtained, provided the knife be sharp. One advantage about the paraffin method is that it is only necessary to have a small part of the blade in proper condition. With celloidin the entire cutting edge must be perfect. Having cut the sections, they should be dropped on the surface of a bowl of warm water (45° C.). This causes the section to flatten out evenly.

Decalcification.—This is best accomplished by fixing in 10 % formalin for twenty-four hours, then placing a small piece of the bone (not exceeding one-half inch square and one-fifth of an inch thick) in concentrated sulphurous acid.

This decalcifies in about twenty-four hours. Wash thoroughly in alkaline water and then in tap water. Pass through alcohols and xylol and imbed and section as before described.

To Stain Sections.—It is first necessary to affix the section to a slide or cover-glass.

To attach the section firmly to the slide, so that it will not become detached in subsequent treatment, pick up a section on a strip of cigarette paper.

A sheet of cigarette paper is cut into about five pieces (1/2 x 1 1/2 inches). Inserting the strip of cigarette paper under the section, it is easily lifted up out of the water. Then apply the slip of cigarette paper, section downward, to a perfectly clean slide. Blot with a piece of filter-paper, then strip off the piece of filter-paper leaving the section smoothly applied to the slide. Next place in the 37° C. incubator for twelve to twenty-four hours and the section will be found to be so firmly attached that it will not be dislodged by subsequent treatment.

For Immediate Diagnosis.—Take a loopful of albumin fixative (white of fresh egg, 50 c.c.; glycerin, 50 c.c.; sodium salicylate, 1 gram) and deposit it on a cover-glass. Now take up a loopful of 30 % alcohol (1 drop of 95 % alcohol and two drops of water) and applying it over the albumin fixative, smear out the mixture uniformly over the cover-glass.

2. Pick up a section on a strip of cigarette paper and apply it to the prepared surface on the cover-glass. Blot with gentle pressure with a piece of filter-paper over the strip of cigarette paper, and strip off this latter, leaving the section attached to the cover-glass.

3. Now, turning the flame of the Bunsen burner down very low or with a small alcohol flame, we hold the cover-glass in a Stewart's forceps, section side up, over the flame and slowly lower it until the paraffin is observed to melt. This shows a temperature of about 50° C. The section is fixed by the coagulation of the albumin at about 70° C. To obtain this temperature lower the cover-glass still more, and the moment vapor is seen to rise from the section it indicates the attachment of the section to the cover-glass.

4. Flood section on cover-glass or slide with xylol; this dissolves out the paraffin. It is better to pour off the first xylol and drop on fresh xylol (one minute).

5. Remove xylol with two applications of absolute alcohol (one minute).

6. Treat specimen with two or three applications of 95 % alcohol (one to two minutes).

7. Next wash in water (one to two minutes).

8. Flood specimen with hæmalum or Delafield's hæmatoxylin (three to seven minutes).

9. Wash in tap water for about two to five minutes until a purplish tinge is developed in the section. The alkali in ordinary tap water develops this color.

10. Apply 1 to 1000 eosin for thirty seconds to one minute.

11. Wash in water; then in 95 % alcohol; then in absolute alcohol.

12. Apply a few drops of xylol and as soon as the section is perfectly transparent mount in balsam.

The staining by hæmatoxylin and eosin is the best for the study of the histology of a section. It only requires about ten minutes to run a preparation through for diagnosis by this method.

The reagents are best kept in dropping-bottles.

The staining of sections on slides is exactly as for those on cover-glasses. Coplin's staining jars are very convenient for use in staining slides.

Where the cover-glass method is used, staining by Gram's method, acid-fast staining, capsule staining, etc., may be carried out as for bacterial preparations.

For staining Gram-positive bacteria in sections, the Gram method as for bacterial preparations, using dilute carbol fuchsin as a counter stain, gives good results.

For Gram-negative bacteria stain with thionin as for blood preparations (ten to twenty minutes). Then differentiate in 1 to 500 acetic acid solution for ten to twenty seconds, wash with water, then with 95 % alcohol, and quickly through absolute alcohol and xylol.

Nicolle's Method.—1. Stain with Löffler's methylene blue ten to fifteen minutes.

2. Differentiate in 1 to 500 acetic acid ten to twenty seconds.

3. Place in 1 % solution of tannin for a few seconds (fixes color).

4. Wash in water, then into 95 % alcohol, absolute alcohol, xylol, and balsam.

Van Giesen's Stain.—Take of 1 % aqueous solution acid fuchsin from 5 to 15 c.c. Saturated aqueous solution picric acid 100 c.c. The method of using is to first stain with hæmatoxylin in the usual way. Then pour on the picro-acid fuchsin solution and allow to stain for one to five minutes. Wash, pass through alcohols and xylol and mount in balsam.

Connective-tissue fibers, axis cylinders, and ganglion cells are stained a bright garnet red. Myelin, muscle fibers, and cells generally are stained yellow. Nuclear staining is that of hæmatoxylin. The stronger stain is used for nerve tissue; the weaker, for demonstrating connective tissue in tumors.

Levaditi's Method.—Take small pieces of tissue, about 2 mm. in thickness, and harden in 10 % formalin for twenty-four hours and then in alcohol for the same period; then wash in water for a short period. They are stained in a freshly made solution of silver nitrate 1.5% for three successive days, changing the solution each day, maintaining the blood temperature, and excluding light. The tissue is then placed in a 2% solution of pyrogallic acid, with the addition of 5% formalin. After remaining in this for twenty-

four hours, light being excluded, they are passed through 85%, 95%, and absolute alcohol, respectively; embedded in paraffin; and cut in about 5 micron sections. Equally good results may be obtained by allowing the silver nitrate to act at room temperature and embedding in celloidin.

Romanowsky.—Staining sections with Romanowsky stains is not very satisfactory. The differential staining seems to fade out in passing through the alcohols. This may be avoided by blotting the section after staining and differentiation and then applying the xylol to the blotted section. After staining with Giemsa's stain for ten to fifteen minutes, differentiate with 1 to 500 acetic acid. When the section has a pinkish tinge, wash in water, dry, clear in xylol, and mount.

Good tissue staining may be gotten with Wright's stain. After removing the paraffin with xylol and the xylol with absolute alcohol, pour on a sufficient number of drops of stain and immediately dilute with an equal number of drops of water. Allow the diluted stain to remain for three to five minutes. Next wash in water, differentiate, until the tissue has a pinkish tinge, in 1 to 500 acetic acid. This differentiation is best done in a tumbler of the dilute acetic acid.

After washing in water, quickly pass through 95% and absolute alcohol, clear in xylol, and mount.

B—MOUNTING AND PRESERVATION OF ANIMAL PARASITES.

To Mount Small Round Worms.—Wash the hook, whip, or filarial worm in salt solution, then drop in 70% alcohol containing 5% of glycerin; the glycerin-alcohol mixture being at a temperature of 60° C. When cool, pour into Petri dishes and allow the alcohol to evaporate in the 37° C. incubator.

Mount in glycerin jelly, preferably in a concave slide, and ring the preparation with gold size. The following is the formula for Kaiser's glycerin jelly: Soak one part of gelatin in six parts of distilled water for two hours. Then add seven parts of glycerin. To the mixture add 1% of carbolic acid, warm for fifteen minutes, with constant stirring, and then filter through cotton.

To Prepare Tape-worms.—Wash in salt solution. Wrap around a piece of glass as a glass slide and fix in salt solution containing 2 to 5% of formalin. Then keep the preparation permanently in 70% alcohol. If preferred, the specimen may be run through alcohols and xylol and mounted in balsam.

Larvæ.—Mosquito larvæ may either be prepared as for small round worms or they may be dropped into 70% alcohol at 60° C. and then passed through alcohols and cleared in xylol and mounted in balsam. Flukes and insects may require treatment with hot (60° to 70° C.) solution of 10 to 20% sodium-hydrate solution. Then wash thoroughly in water and subsequently pass through alcohols to xylol and mount in balsam. Clove oil or cedar oil clears more slowly, but makes specimens less brittle than does xylol. Another satisfactory method is to drop insects or larvæ into acetone at 60° C. and after being in this from one to twelve hours to clear in xylol or clove oil and mount in balsam.

Looss has a method of first washing a small nematode or delicate fluke in salt solution. Then pouring this first salt solution out of the test-tube in which the washing was carried out, to add fresh salt solution, and then an equal amount of saturated

aqueous solution of bichloride of mercury. The shaking is easily carried on in the test-tube. After washing in water the worm is passed through alcohols, one strength of which should contain iodine. Clear in xylol and mount in balsam.

A quick method of preparing small nematodes for examination is to fix them for from two to twelve hours in 5 to 10 % formalin, this being heated to 60° C. at the time the worms are dropped into it. Then transfer to the following solution:

Glucose syrup (glucose, 48; water, 52),	100 c.c.
Methyl alcohol,	20 c.c.
Glycerine,	10 c.c.
Camphor, q.s. (a small lump for preservation).	

They may be mounted directly in this and the cover-slip ringed with about 60° C. paraffin, followed with gold size.

Preparations so cleared and mounted in glycerin jelly should also be ringed with paraffin or some cement.

Flukes, cestodes, and nematodes are best stained with carmine. The following is a good formula.

Dissolve, by boiling, 4 gm. carmine in thirty drops HCl and 15 c.c. water. Then add 95 c.c. of 85 % alcohol and filter while hot. Neutralize with ammonia until precipitate begins to form. Then filter cold.

1. Stain parasites taken from 70 % alcohol for five to twenty minutes. 2. Differentiate in 3 % hydrochloric acid. 3. Pass through alcohols to xylol and mount in balsam.

To Prepare Flies or Mosquitoes for Transmission Through the Mails.—Wrap the insect carefully in a piece of tissue paper (toilet-paper answers). Impregnate sawdust with 5 % carbolic acid solution and fill around the folded insects in the box containing them. (Barely moisten.)

C—PREPARATION OF NORMAL SOLUTIONS.

A normal solution is one which contains the hydrogen equivalent of an element, expressed in grams, dissolved in sufficient distilled water to make 1000 c.c. The hydrogen equivalent is the atomic weight of any element divided by its valence. In a base, salt or acid, we use the molecular weight in grams divided by valence.

What may be considered as the valence of a base is shown by the number of hydroxyls combined with it; that of an acid by the number of replaceable hydrogen atoms which it contains.

To make a normal solution, dissolve in distilled water a weight in grams equal to the sum of the atomic weights of the substance divided by its valence, and make up the volume to exactly 1000 c.c.

NaOH is univalent. Na = 23. O = 16. H = 1. Dissolve 40 grams NaOH in water and make up to exactly 1000 c.c.

Oxalic acid is $COOH-COOH+2H_2O$ which gives it a molecular weight of 126. As it contains two carboxyl groups it is dibasic, and it is necessary to divide the molecular weight by 2, so that for a normal solution of oxalic acid we dissolve 63 grams in a volume of distilled water made up to 1000 c.c.

If a chemical laboratory is not accessible one may prepare normal solutions with an error so slight as to be unimportant in clinical work in the following way:

Sodium hydrate being very hygroscopic, it is impossible to accurately prepare a normal solution by directly weighing out the substance. Instead, select perfect crystals of oxalic acid, such as can be obtained in a drug store, and weigh out on the most accurate apothecary scales obtainable exactly 6.3 grams of the most perfect crystals in the bottle. Put these preferably in a volumetric flask and make up with distilled water to 1000 c.c. Less accurate is the use of a measuring cylinder. If care is used this should give N/10 solution of oxalic acid in which the error is less than 1 %.

Having N/10 acid at hand, we may prepare N/10 NaOH in the following way: Weigh out an excess of sodium hydrate (5 grams of stick caustic soda) and dissolve in 1100 c.c. of distilled water. Take up 10 c.c. of this solution with a pipette and let it run into a beaker. Add six drops of phenolphthalein solution. This gives a violet-pink color. Fill the burette with the N/10 oxalic-acid solution and let it run into the sodium-hydrate solution in the beaker until the pink is just discharged. Reading off the number of c.c. of the N/10 acid used, we know the strength of the sodium-hydrate solution. It is well to repeat the titration and take an average.

If 10.5 c.c. of the oxalic-acid solution were required it would show that the sodium hydrate solution was stronger than N/10, as only 10 c.c. would have been necessary if the NaOH solution had been N/10. It is therefore necessary to dilute the sodium-hydrate solution in the proportion of 10 to 10.5. Measure exactly 1000 c.c. of the too concentrated sodium-hydrate solution and add to it 50 c.c. of distilled water, mix thoroughly, and we have 1050 c.c. of N/10 solution of NaOH. $1000 \times 10.5 = 10{,}500$. $10{,}500 \div 10 = 1{,}050$.

As Acidum hydrochloricum U. S. P. is about two-thirds water (68.1 %) to make N/10 HCl, which would require 3.65 in 1000 c.c., it would be necessary to take about three times this amount of U. S. P. acid. Take 12 c.c. of the acid and add distilled water to make 1100 c.c. Put 10 c.c. of this dilute solution in a beaker. Add phenolphthalein solution and titrate. If 11 c.c. of N/10 NaOH were required it would be necessary to add 100 c.c. of water to a volume of 1000 c.c. of the diluted hydrochloric acid. $1000 \times 11 = 11000 \div 10 = 1100$.

Other acid and alkali solutions can ke made as for N/10 HCl and N/10 NaOH.

D—DISEASES OF UNKNOWN OR NOT DEFINITELY DETERMINED ETIOLOGY.

Of Temperate Climates.

Acute Articular Rheumatism.—Various bacteria have been reported as cause.

Epidemic Poliomyelitis.—Virus unknown but material from the cord of child with the disease when injected subdurally, intravascularly, or into the peritoneal cavity of monkeys produced the disease in the animals inoculated. The virus has been passed through three generations of monkeys (Flexner).

Foot-and-mouth Disease.—Probably due to an ultramicroscopic organism.

Measles.—Cause entirely unknown. Hektoen has shown that blood contains the virus.

Mumps.—Herb has implicated a diplococcus. Inoculations into Steno's duct of monkeys successful.

Rabies.—Probably the Negri bodies.

Roetheln (German Measles).—Nothing known.

Scarlet Fever.—Streptococci seem most probable cause (S. anginosus). Mallory has implicated epithelial protozoa.

Small-pox and Vaccinia.—Guarnieri and Councilman have implicated epithelial protozoa.

Spotted Fever of the Rocky Mountains.—Supposed to be due to an unknown protozoon transmitted by a tick.

Typhus Fever.—It has been suggested that the cause may be a protozoon transmitted by vermin.

Recent work by Anderson and Ricketts has shown that the blood of human cases is infective for monkeys. The virus does not seem to pass through a Berkefeld filter and the epidemiology points to the body louse as the transmitting agent.

Varicella.—Entirely unknown.

Whooping Cough.—Influenza-like bacilli have been implicated.

Of Tropical Climates.

Ainhum.—(A disease characterized by a constricting fibrous ring, especially of little toe, often leading to spontaneous amputation.)

Beriberi.—Various microorganisms and food factors suggested.

Blackwater Fever.—Considered as a malarial disease, but thought by some to be possibly caused by a protozoon—a Babesia (Piroplasma).

Dengue.—Supposed to be due to a protozoon transmitted by Culex fatigans.

Goundou.—(Symmetrical bony tumors of nasal processes of superior maxillary bones.)

Sprue.—(A form of chronic diarrhœa characterized by diaphanous thinning of gut and ulcerations of buccal cavity.)

Tsutsugamushi.—(A disease of Japan somewhat resembling typhus fever.) Supposed to be due to a protozoon transmitted by the Kedani mite.

Yellow Fever.—Supposed to be due to a protozoon transmitted by the Stegomyia calopus.

E—CHEMICAL EXAMINATION OF URINE.

For the prevention of decomposition when a urine is not examined shortly after voiding, chloroform (10 to 20 drops added to a tightly corked bottle) or formalin (4 or 5 drops to a pint of urine) are ordinarily employed. Formalin is better for microscopical material, but, owing to its reducing power, should be substituted by boric acid in urine to be examined for sugar. For clearing urine, rubbing up with Talcum purificat. U. S. P. and filtering is recommended.

A twenty-four-hour specimen is necessary for accurate work. The urine should

be collected in clean separate bottles. Where pus comes from the bladder the proportion of pus in each bottle will be practically the same; if from the kidneys the amount will vary in the different bottles.

The amount of urine varies in different individuals (water or beer habit). It is usually given as from 1000 to 1500 c.c.

Long proposes to substitute 2.6 for Hæser's coefficient which, if multiplied by the two final figures of the specific gravity taken at 25° C., gives the weight of urinary solids in 1000 c.c.

Albumin.—Practically serum albumin alone is clinically important.

The two usual tests are 1. Heat test and 2. Heller's nitric acid test. For the former, add 3 to 10 drops of 5 % acetic acid to the perfectly clear urine in a test-tube and bring to a boil. By boiling the upper portion a turbidity in contrast with the clear lower portion may be obtained.

For Heller's test, pour a small amount of nitric acid into a narrow test-tube and, while holding the tube at an angle of about 45°, superimpose a layer of the urine to be tested, which is delivered drop by drop from a pipette and allowed to flow down the side of the tube.

This test can be converted into a quantitative one which is sufficiently accurate for clinical purposes. It is based on the fact that a specimen of urine containing .003 % of albumin will give a perceptible ring at the layering of the urine and acid in two minutes. If the ring appears at once or in a few seconds the albumin content is greater. From the qualitative test an idea can be formed as to the amount of albumin which the urine contains, a heavy ring forming immediately showing a considerable albumin contents Probably the highest elimination of albumin is found in chronic parenchymatou. nephritis where it may run from 1 to 3 %. In an ordinary case of acute nephritis .5 % would be an average content.

Pour into a series of four or five small test-tubes a small amount of nitric acid and mark them 1, 2, 3, 4, etc., with a blue pencil. In an ordinary graduated cylinder make a greater or less dilution according to the content roughly estimated in the qualitative test. Using a 50 c.c. cylinder, dilute 10 c.c. urine to 50 c.c. with water. Mix and draw off with a pipette a few c.c. of this 1 to 5 dilution. Superimpose this urine on the nitric acid in tube 1. If a ring is formed at once pour off from the cylinder the 1 to 5 diluted urine so that only 10 c.c. remain. Dilute this remaining diluted urine with water to 50, giving a 1 to 25 dilution. Test this in tube No. 2 for the ring. If again the ring forms in a few seconds make a third dilution (1 to 5) as given above, and again test. A faint but distinct turbid ring forming in practically two minutes shows that the urine as diluted contained .003 % of albumin. In the above example the first diluted urine tested was 1 to 5, the second 1 to 25 and the third 1 to 125. This final and successful dilution multipled by .003 gives the percentage of albumin in the urine. ($125 \times .003 = .375$ %.)

If it is desired to determine the nature of the albumins present in the urine, the following scheme is simple. Acidify the urine and filter until perfectly clear, use acetic acid as for the boiling test. A small portion of urine is largely diluted with water. On standing precipitate shows nucleo-albumin. To a saturated solution of

sodium chloride in a test-tube add an equal quantity of the acidified urine—a precipitate shows albumose. Filter off and boil the filtrate—a precipitate shows serum albumin.

To test for serum globulin add a drop or so of the urine to distilled water in a beaker. A milky cloudiness shows globulins, these being insoluble in distilled water.

As a test for the *Bence-Jones body*, usually present in multiple myelomata, that of Boston is of value. Mix 15 c.c. urine in a test-tube with an equal amount of saturated NaCl solution. Add 2 c.c. of 40% NaOH solution and shake the contents of the tube thoroughly. Heat the upper contents of the tube to boiling and add lead acetate solution (10%) drop by drop continuing the heating. A brown to black precipitate (sulphur) shows this form of albumin.

In tests requiring the removal of albumin boil the urine and add dilute acetic acid until the precipitate is flocculent, then filter.

SUGAR.

Fehling.—Pour equal parts of Fehling's copper solution (34.639 grams of copper sulphate in 500 c.c. of water) and Fehling's alkali solution (173 grams sodium potassium tartrate and 50 grams sodium hydrate in 500 c.c. water) into a test-tube. Mix and dilute the deep blue solution with four parts of water. Heat the upper portion of the diluted Fehling's solution in the flame to boiling and drop in from a pipette the urine to be examined. A yellowish to red precipitate shows the presence of sugar.

Phenylhydrazin (Kowarsky).—Mix five drops of pure phenylhydrazin in a test-tube with ten drops of glacial acetic acid. Shake lightly and add 15 drops of saturated solution of NaCl. This makes a pasty mixture. Now add 10 c.c. of the urine and bring carefully to a boil over a small flame and continue to boil gently for two minutes. Upon cooling a yellowish crystalline precipitate falls more or less rapidly according to the sugar content of the urine. If the urine contains .2% or more of sugar the precipitate appears in a few minutes. The test is sensitive for .03% of sugar.

Fermentation Test.—This is the surest test for sugar in the urine. It will show the presence of .05% of glucose. Instead of the Einhorn apparatus one may be extemporized by taking a 50 c.c. cylinder, filling it to overflowing with the urine which has previously been rubbed up with a piece of compressed yeast the size of a hazel-nut. The urine should be made acid with tartaric acid. A small 3-in. test-tube is filled with the yeast-treated urine and dropped mouth downward into the 50 c.c. cylinder. The apparatus is incubated for twenty-four hours and the presence of gas in the closed end of the test-tube shows that sugar was present. A control to determine that the yeast does not contain sugar is advisable. To utilize this test as a quantitive one, first accurately take the specific gravity of the urine; then add the yeast and fill the test-tube and cylinder as directed above. Next pour off or pipette off the urine exactly to the 50 c.c. mark. Incubate for twenty-four to forty-eight hours and make up the loss by evaporation, with distilled water. After the urine has cooled down to room temperature the contents of tube and cylinder are thoroughly mixed (the small tube having been withdrawn with a pair of forceps) and the specific gravity again taken and the difference between this and the first reading multiplied by .23. Example: Specific gravity of unfermented urine, 1.030, that of urine after incubation, 1.022. Difference, $8 \times .23 = 1.84\%$.

Benedict's Method for the Quantitative Determintion of Sugar.

In this method reduced copper is thrown down as a white precipitate of cuprous sulphocyanate.

Three solutions are necessary:

Solution A.	Crystallized copper sulphate,	69.3	gm.
	Distilled water to	1000.	c.c.
Solution B.	Pure Rochelle salt,	346.	gm.
	Anhydrous sodium carbonate,	200.	gm.
	Distilled water to	1000.	c.c.
Solution C.	Potassium sulphocyanate,	200.	gm.
	Distilled water to	1000.	c.c.

Mix 10 c.c. each of the three solutions. The 30 c.c. of the mixture are equivalent to .073 gm. glucose.

Fill a burette with the urine to be tested or preferably treated in the following way. Shake up 50 c.c. urine with 3 grams basic lead acetate and filter. Treat filtrate with 3 grams neutral potassium oxalate, stir and filter. Fill the burette with the cleared urine.

Introduce the 30 c.c. of mixed A. B. and C. solutions into an Erlenmeyer flask, add 2 or 3 grams of anhydrous or 5 grams of crystallized sodium carbonate and bring the mixture in the flask to a boil. A small piece of pure, well washed absorbent cotton in the flask will prevent bumping during the titration. As soon as the sodium carbonate is in solution, run in the urine from the burette rather rapidly until a heavy chalky white precipitate is formed; then more slowly and carefully until the last trace of blue color disappears. The end reaction is sharp. The number of c.c. of urine used contains .073 gram glucose. Example: Required 6 c.c. urine; then $100 \times .073 = 7.3 \div 6 = 1.21\%$ of sugar.

The Pancreatic Reaction of Cammidge in the Urine.

Cammidge claims that there is a definite and important relationship between his pancreatic reaction in the urine and disease of the pancreas. The results of other workers go to support this view, particularly when considered in connection with the examination of the fæces for neutral fat.

The principle of the reaction depends upon the formation in the urine of a substance having the characters of an unfermentable pentose sugar after boiling with hydrochloric acid. It is not present in the original urine as such, and forms an osazone on treatment with phenylhydrazine, easily distinguished from the corresponding compound of glucose. As the presence of glucose would seriously interfere with the success of the reaction, all specimens of the urine examined must be carefully tested for glucose, and, if present, it must be removed by fermentation with yeast cake. Glucose is rarely present.

The technic of the reaction requires considerable time, but is easy of manipulation, and should be readily carried out in any hospital. The urine, if alkaline, must be made acid in reaction, and any albumin or sugar present must be removed and the urine made

up to its original bulk with distilled water. To 40 c.c. of the clear filtered urine are added 2 c.c. of concentrated hydrochloric acid, and the mixture gently boiled for ten minutes in a small flask, using a funnel in the neck as a condenser. It is now cooled and distilled water added to again make up the contents to 40 c.c., owing to the loss by evaporation. Eight grams of lead carbonate are now slowly added to neutralize the excess of acid. After standing for a few minutes the flask is again thoroughly cooled and the contents filtered until perfectly clear. The filtrate is then well shaken with 8 grams of powdered tribasic lead acetate, and the resulting precipitate removed by filtration, which is repeated until perfectly clear.

The excess of lead in solution must now be removed by treating with 4 grams of powdered sodium sulphate; the mixture is heated to boiling, then thoroughly cooled and filtered. From the filtrate are measured 17 c.c.; this is transferred to a small flask with funnel condenser and there are added 2 grams of sodium acetate, 0.8 grams phenylhydrazine hydrochloride and 1 c.c. of 50% acetic acid. The mixture is then boiled gently for ten minutes, filtered into a test-tube with a mark showing 15 c.c., and made up, if necessary, to that point with hot distilled water. The filtrate is carefully stirred and left to stand over night.

The quantity and time of deposit of the crystals will depend upon the degree of extension of the inflammatory process in the pancreas. Thus, in well-marked cases, a light-yellow flocculent precipitate should appear in a few hours, but in less characteristic cases it may be necessary to leave the preparation over night before a deposit occurs. Under the microscope the precipitate is seen to consist of long, light-yellow, flexible, hair-like crystals of pentosazon, arranged in delicate sheaves.

Urinary Tests in Connection with Acidosis.

The determination of the ammonia quotient, which is the ratio of N eliminated as ammonia to total nitrogen elimination, has assumed great importance by reason of its connection with various forms of acid intoxication, as in diabetes, pernicious vomiting of pregnancy, and various hepatic diseases.

The degree of acidosis is better determined by the quantitative estimation of nitrogen elimination as ammonia than by estimating quantitatively the amount of diacetic and β-oxybutyric acid in the urine. Normally we have about .7 gram of ammonia eliminated daily. In acidosis this may rise to 5 or 10 grams and instead of being from 3 to 5% of the total N, it may amount to 30 to 50%.

Formalin Method for the Estimation of Ammonia.

Free ammonia reacts with formalin to form hexamethylenetetramine. If sodium hydrate is added to neutralized urine in the presence of formalin free ammonia is liberated and reacts with the formalin. So soon as all the ammonia has been liberated, the end reaction occurs. The technic is as follows: About 60 c.c. of filtered urine are treated with 3 grams of basic lead acetate, well stirred, allowed to stand a few minutes and filtered. The filtrate is treated with 2 grams of neutral potassium oxalate well stirred and filtered; 10 c.c. of the clear filtrate are diluted to 50 c.c. with distilled water; a few drops of 1% phenolphthalein solution are added. The mixture will be slightly

alkaline or acid. Fifteen grams potassium oxalate are added and stirred. It is exactly neutralized with decinormal NaOH or H_2SO_4. Twenty c.c. of 20% commercial formalin, previously made neutral, are added, and the solution again titrated with decinormal NaOH to neutralization. Every c.c. of decinormal NaOH corresponds to 0.0017 gram NH_3. The quantity of ammonia is then calculated on the basis of the twenty-four-hour volume. Example: The 10 c.c. of urine required 4 c.c. N/10 NaOH to give a pink color. $4 \times .0017 = .0068$. Then 100 c.c. urine would contain .068 and 1000 c.c. (twenty-four-hour urine amount) .68 gram of ammonia.

ESTIMATION OF TOTAL NITROGEN.

Technic.—Solutions required:

1. Twenty per cent. commercial formalin previously made neutral with NaOH.
2. N/10 NaOH.
3. Forty per cent. NaOH.

Ten c.c. of filtered urine are pipetted into a Kjeldahl or Koch flask; 10 c.c. of concentrated H_2SO_4 and 10 grams K_2SO_4 are added. The mixture is heated over a free flame, gently at first to avoid foaming, and is finally brought to a boil, which is continued until the mixture is perfectly clear, usually requiring forty-five minutes to an hour, The contents are cooled and quantitatively transferred to a 200 c.c. volumetric flask and 1 c.c. of phenolphthalein solution added. The greater part of the acidity is now neutralized by adding about 30 c.c. of the 40% NaOH. It is cooled under a water tap and made up to the 200 c.c. mark; 10 c.c. are taken, diluted to 50 c.c. with distilled water and exactly neutralized with N/10 NaOH. Twenty c.c. of the formalin solution are now added and the titration again performed. The pink end reaction is beautifully clear and sharp. The second reading multiplied by the factor 0.0014 gives the amount of nitrogen in grams in 10 c.c. of the fluid. It is then computed for the twenty-four-hour volume as for N, eliminated as ammonia. Example: It required 5 c.c. N/10 NaOH—$5 \times .0014 = .007$. As original 10 c.c. were diluted to 200, the 10 c.c. taken for titration would only be 1/20; hence $.007 \times 20 = .14$ gram for 10 c.c. or 1.4 for 100 c.c. or 14 grams for 1000 c.c.

The amount of urea, which represents from 85 to 90% of the total nitrogen, is usually determined instead of the total N. The hypobromite and hypochlorite methods are, however, lacking in accuracy, and more exact methods of urea estimation are more time-consuming than the one just given for total N.

Gerhardt's Test for Diacetic Acid.

Add a few drops of ferric chloride solution to 10 to 50 c.c. of urine so long as a precipitate continues to form. Then filter and to the filtrate add more ferric chloride solution. A bordeaux red color shows diacetic acid. The test is sensitive. As a control to show that the color is not due to drug elimination (antipyrine, salicylates, etc.) boil a specimen which gave the test for three to five minutes. If the color was due to drugs it will be obtained with a boiled sample while such treatment drives off the diacetic acid.

It has been recommended, as being more sensitive, to add the urine to the ferric chloride solution instead of the usual method of adding the iron solution to the urine.

If the urine shows a well-marked Gerhardt reaction it is well to test for β-oxybutyric acid.

The following modification of Lange's test by Hart is a satisfactory one. The principle involved is the removal of acetone and diacetic acid by heat, then oxidizing β-oxybutyric acid to acetone with hydrogen peroxide and then testing for acetone.

Method: Take 20 c.c. of urine, dilute with an equal amount of water and add a few drops of acetic acid. Next boil in a beaker until the original amount of diluted urine is reduced to 10 c.c. (originally 40 c.c.). Dilute this evaporated urine with an equal amount of water, giving us 20 c.c. In each of two test-tubes put 10 c.c. of this 20 c.c. To one tube add 1 c.c. of hydrogen peroxide and warm gently, without boiling, for one minute; then cool. The other tube is left untreated. Next, to each test-tube add 10 drops of glacial acetic acid and 5 or 10 drops of a freshly prepared sodium nitroprusside solution and mix. Next carefully overlay each tube with about 2 c.c. of concentrated ammonia. If β-oxybutyric acid were present in the tube treated with the hydrogen peroxide and thereby oxidized to acetone a violet-red ring will develop at the point of contact while in the untreated tube there will be no such color ring.

A yellowish-brown ring from the presence of creatinin may show in the untreated tube. It is well to allow the tubes to stand for three to four hours before finally reporting the absence of β-oxybutyric acid. It will probably show .2%.

Indican.—To a test-tube one-fourth full of urine add an equal volume of concentrated hydrochloric acid and 3 to 4 c.c. of chloroform and 2 to 3 drops of a 2% solution of potassium permanganate. Gently mix the contents avoiding violent shaking (emulsification of chloroform). The chloroform at the bottom of the tube will be colored a light or deep blue according to the amount of indican present.

A more exact method is to pour off the supernatant acid urine. Wash the chloroform with water, then pour off as much of the supernatant water as possible and add 10 c.c. of alcohol. A clear blue fluid results.

Urobilin.—Urobilin appears in considerable quantity in urine when there is much destruction of red cells, as in pernicious anæmia, internal hæmorrhage, and in malaria cachexia. The best test is that of Schlesinger. To the unfiltered urine add an equal amount of a saturated solution of zinc acetate in absolute alcohol. Shake, add a few drops of Lugol's solution and filter. Fluorescence in the filtrate shows the presence of urobilin. The degree of blood destruction is indicated by the intensity of the fluorescence.

Bile Pigments.—A satisfactory test is that of Rosin (Trousseau). Overlay 10 c.c urine with about 5 c.c. of tincture of iodine (1 to 10 of 95% alcohol). An emerald green ring at the point of contact shows the presence of bile coloring matter.

F—CHEMICAL EXAMINATION OF GASTRIC CONTENTS.

The test breakfast ordinarily used is that of Ewald (one shredded wheat biscuit or two small pieces of toast with 400 c.c. of water is what is usually given). This Ewald breakfast is a low-grade stimulant to acid production. It is given in the morning on an empty stomach. If at supper, the night before, the patient partake of raspberry jam the finding of the characteristic seeds in the stomach contents the next morning would

be evidence of lack of motor activity. The Fischer meal which contains a 4-ounce Hamburg steak in addition to the water and toast of the Ewald is withdrawn after three hours.

The stomach tube is more easily passed if it be thoroughly chilled in ice water without the use of any lubricant.

The stomach tube should be passed one hour after the Ewald breakfast and if more than 50 c.c. of fluid be obtained it indicates stasis or hypersecretion.

Filter the gastric contents and test first for free HCl. The most reliable and sensitive test is that of Gunsberg. The reagent, which should be freshly prepared, consists of phloroglucin 2 grams, vanillin 1 gram, and absolute alcohol 30 c.c. By mixing 2 drops of gastric juice and an equal quantity of Gunsberg reagent in a small porcelain dish and carefully heating above a flame we obtain a carmine red color if free HCl be present.

For lactic acid a modification of Strauss' method is quite satisfactory. Shake, in a test-tube, 5 c.c. of gastric contents with 20 c.c. of ether, allow to settle and pour off 5 c.c. of the supernatant ether into another test-tube. To this ether add 20 c.c. of water and 2 drops of a 1 to 9 solution of ferric chloride and shake well. The presence of 1% of lactic acid will give an intense greenish color.

Having determined the presence or absence of free hydrochloric or lactic acid, we should make a quantitative test of the various factors producing the acidity of gastric juice (a modified Töpfer test). These are: 1. Free HCl. 2. Combined HCl. 3. Acid salts, and 4. Total acidity.

To 10 c.c. of filtered gastric contents, in a beaker, add 3 drops of dimethyl-amido-azo-benzol solution (a 1/2% solution in 95% alcohol). In the presence of free HCl the fluid becomes a rich carmine pink.

After reading the burette run in N/10 NaOH solution until the pink color is discharged and a light yellow color is obtained. This reading multiplied by 10 gives the amount of free HCl in degrees, a degree corresponding to 1 c.c. N/10 NaOH. Next add 6 drops of a 1/2% alcoholic solution of phenolphthalein to the light yellow fluid in the beaker. Again titrating the same preparations we add N/10 NaOH until a faint but distinct pink color is produced. The number of c.c. added for the free HCl plus the number to give the pink color when multiplied by 10 gives the total acidity in degrees. (For example: 2.5 c.c. N/10 NaOH used to obtain yellow color—$2.5 \times 10 = 25$ or acidity due to free HCl. After adding the phenol-phthalein, 4 c.c. N/10 NaOH required to produce pink color—$4 + 2.5 \times 10 = 65$ or total acidity in terms of acidity. This means that it would require 65 c.c. N/10 NaOH to neutralize 100 c.c. of gastric juice. A total acidity of 60 is about normal. To obtain percentage in HCl multiply by .00365; thus, $65 \times .00365 = .23$ HCl.)

Having determined the total acidity add 3 c.c. of 10% neutral calcium chloride solution to the gastric contents already in the beaker. As a result of the formation of acid calcium phosphate the pink color is discharged. Again add N/10 NaOH from the burette until the pink color is restored. The number of c.c. used gives the amount of acid salts present.

From the figures for the total acidity subtract the sum of that for free HCl and for acid salts and the remainder will give the acidity due to combined HCl.

G. HERMAN'S STAIN FOR TUBERCLE BACILLI.

It has been claimed that this stain gives better satisfaction than the Ziehl-Neelsen. It consists of two solutions: (1) ammonium carbonate in distilled water, 1%; (2) crystal violet (methyl violet 6B) in 95% ethyl alcohol, 3%. The two solutions are kept in separate bottles and, for staining, one part of (2) is mixed with three parts of (1). The sections are placed on a cover-glass, the water evaporated, and about seven drops of the staining mixture are placed on the specimen and allowed to steam for one minute over a water-bath. Place for a few seconds in 10% nitric acid and then in 95% alcohol to decolorize. Mount without a counterstain or use eosin 1% or a very dilute fuchsin. The organisms are purple. This staining method may be applied to smears of concentrated or unconcentrated sputum in the same manner as for sections of tissue.

H. TUBERCLE BACILLI.

Tubercle bacilli usually occur nested in clumps of sputum. Therefore, when few in number it is only by chance that they may be found. Concentration methods aim to dissolve these clumps of sputum and collect, free from mucus, whatever bacilli may be present. There are many concentration methods for sputum. One of these has been given above. Uhlenhuth's method has some advantages over others in the solvent used: 1. It breaks up the sputum very rapidly; 2. it immediately dissolves all organisms except acid-fast ones; 3. applied in not too concentrated form and for not too long a time, tubercle bacilli are not killed, so that by washing the sediment carefully by several dilutions and centrifugings we have in the sediment viable tubercle bacilli which we may attempt to cultivate upon Dorset's or other suitable media with the reasonable hope that contaminations will not choke them out or prematurely kill the inoculated guinea-pig; 4. it has less effect upon the staining properties of tubercle bacilli than any other material used in concentration methods.

This solvent is an alkaline Javelle water. It may be purchased in the market under the name of antiformin and is made as follows:

Take 908 grams of bleaching powder and 3 liters of water, and to each 180 grams of the bleaching powder add 65 grams of sodium carbonate. Mix thoroughly. Allow this to stand over night. Filter and test filtrate for chlorine (KI and hyposulphite). There should be about 5.4% of available chlorine. To this filtrate add 7.5% of sodium hydrate and filter. This last filtrate is the antiformin.

To simply concentrate for demonstrating tubercle bacilli, mix the sputum with that quantity of antiformin which trial shows is sufficient to liquefy it. We may shake this mixture by hand or with a shaking machine hurry the fluidification. Ordinarily it takes only a few moments. When fluid we may either dilute with water and centrifuge (getting rid of alkali which interferes with fixation of the sediment) or we may mix the fluid sputum with some oil such as ligroin or a mixture of petroleum ether and xylol (sp. gr. 0.72) and, having thoroughly shaken this last mixture, again centrifuge. In this case we float the concentrated bacilli up and they are found in smears made from the layer between the two liquids. In this latter case it may be necessary to use *some albumin* fixative with the concentrate, as fixation after the oil is rather difficult. *Stain by Ziehl*-Neelsen's or Herman's method.

In growing bacilli from the sputum or for animal inoculation Uhlenhuth's method is as follows:

He usually takes a 15% sputum-antiformin mixture, though weaker mixtures may be used according to the consistency of the sputum. For example, 10 c.c. of sputum are mixed with 10 c.c. of a 10% antiformin solution. The whole is poured into a Petri dish and stirred thoroughly with a stout platinum loop until it is made as homogeneous as possible; this usually takes half an hour. Then 10 to 20 c.c. are centrifuged thirty minutes, the supernatant fluid withdrawn, and the sediment washed twice in sterile normal salt solution. Of this sediment 4 to 5 loopfuls are then streaked on a serum tube (or glycerine serum). Altogether 6 to 8 tubes are used for each sputum and the coagulation of the medium must have been carefully carried out in an atmosphere saturated with steam. The tubes, after inoculation, are sealed with paraffin, wrapped in dark paper to protect them from light, and incubated in an incubator having a damp atmosphere.

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Cubic Meter.—Unit of space for the number of organisms in air. It contains 1000 liters. It is equal to 1.308 cubic yards or 35.316 cubic feet. One thousand cubic feet, the unit of space in disinfection, is equal to 28.3+ cubic meters.

Liter.—Unit of space for normal volumetric solutions. It contains 1000 cubic centimeters. It is equal to 1.0567 quarts or 33.8+ ounces. A liter of distilled water weighs 1 kilogram.

Cubic Centimeter.—Unit of space for organisms in water, milk, vaccines, etc., 1 c.c.=0.27 fl. dr. There are, approximately, 16 drops in 1 c.c.

Cubic Millimeter.—Unit of space for blood-cells. There are 1000 cubic millimeters in 1 cubic centimeter and 1 million cubic millimeters in 1 liter. In water analysis, as there are 1 million milligrams in one liter, parts in the million and milligrammes per liter are the same.

1 Meter=39.37 inches.
1 Centimeter=.3937 inch. Approximately, 2/5 inch.
1 Millimeter=.0393 inch. Approximately, 1/25 inch.
1 Kilogram=2.2+ pounds av.
1 Gram=15.432 grains.
1 Milligram=0.0154 grain. Approximately, 1/64 grain.
A pound avoirdupois is equal to 453.59 gm.

One hundred cubic centimeters of a saturated solution contains:

	Water	Alcohol
Methylene blue,	6.68	0.66 gram.
Gentian violet,	1.75	4.42 grams.
Basic fuchsin,	0.66	2.92 grams.

FASCIOLA HEPATICA 150 x 80 μ

FASCIOLOPSIS BUSKI 125 x 75 μ

SCHISTOSOMUM HAEMATOBIUM 100 x 50 μ

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